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TITLE:

Assembly of Gold Nanorods into Chiral Plasmonic Metamolecules Using DNA Origami Templates

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SUMMARY:

We describe the detailed protocol for the DNA origami-based assembly of gold nanorods into chiral plasmonic metamolecules with strong chiroptical responses. The protocol is not limited to chiral configurations and can be easily adapted for the fabrication of various plasmonic architectures.

ABSTRACT:

The inherent addressability of DNA origami structures makes them ideal templates for the arrangement of metal nanoparticles into complex plasmonic nanostructures. The high spatial precision of a DNA origami-templated assembly allows controlling the coupling between plasmonic resonances of individual particles and enables tailoring optical properties of the constructed nanostructures. Recently, chiral plasmonic systems attracted a lot of attention due to the strong correlation between the spatial configuration of plasmonic assemblies and their optical responses (e.g., circular dichroism [CD]). In this protocol, we describe the whole workflow for the generation of DNA origami-based chiral assemblies of gold nanorods (AuNRs). The protocol includes a detailed description of the design principles and experimental procedures for the fabrication of DNA origami templates, the synthesis of AuNRs, and the assembly of origami-AuNR structures. In addition, the characterization of structures using transmission electron microscopy (TEM) and CD spectroscopy is included. The described protocol is not limited to chiral configurations and can be adapted for the construction of various plasmonic architectures.

INTRODUCTION:

DNA nanostructures, DNA origami in particular, have been widely used to arrange molecules and other nanoscale components (e.g., proteins and nanoparticles [NPs]), with nanometer precision into almost arbitrary geometries[1–5]. The ability to arrange metal NPs on DNA origami templates with a high yield and accuracy enables the fabrication of plasmonic structures with novel optical properties[6–10]. DNA origami technique is especially useful for the generation of chiral plasmonic structures, which require genuinely three-dimensional architectures[11–20].

This protocol describes in detail the entire process of the fabrication of DNA origami-templated chiral assemblies of AuNRs. The software used for the design[21] and structure prediction[22,23] of DNA origami is intuitive and freely available. The origami fabrication and AuNR synthesis use common biochemistry lab equipment (e.g., thermocyclers, gel electrophoresis, hot plates, centrifuges). The structures are characterized using standard TEM and CD spectroscopy.

The fabrication of similar plasmonic nanostructures with top-down methods (e.g., electron beam lithography) would require rather complicated and expensive equipment. In addition, DNA origami templates provide the possibility to incorporate structural reconfigurability in plasmonic assemblies[24–33], which is extremely challenging for structures fabricated with lithography techniques. Compared to other molecular-based approaches[34–37], DNA origami-based fabrication provides a high level of spatial precision and programmability.

PROTOCOL:

1. Design of the DNA origami

1.1. Identify the desired relative spatial arrangement of AuNRs and the suitable shape of the DNA origami template (**Figure 1A**). Estimate the structural parameters of the AuNRs and the origami templates. Locate the approximate positions of staples that need further modification (**Figure 1B**).

1.2. Download and install caDNAno[18] to design a DNA origami template. In caDNAno, route the scaffold strand according to the desired shape of the template and generate the staple strands by clicking **Seq Tool**. Click **Paint Tool** and mark the staple strands that require further modification (**Figure 1C**).

1.3. Click **Export Tool** to export the DNA staple sequences (**Figure 1C**) to a .csv file.

1.4. Design double-stranded locks to fix the angle *Θ* between the two origami bundles. Depending on the relative orientation of the two bundles, the origami construct can adapt left- or right-handed (LH/RH) chiral spatial configuration (**Figure 1B**).

1.5. Import the staples' .csv file into a spreadsheet application. Add a polyA$_{10}$ sequence at the end of the staples used for AuNR assembly (handles). Modify the staple strands on the designed lock sites with lock sequences.

NOTE: The assemblies in the representative results contain 36 handles protruding at the 3' end of the staple strands, 18 on each DNA origami bundle, equally distributed on two parallel helices every 21 nt. The distance between the first and the last handle position is 168 nt, approximately 57 nm (see the attached caDNAno file).

2. Assembly of the DNA origami templates

2.1. Prepare a working stock of staple stands (SM), including strands with handles and locks, by mixing equal amounts of concentration-normalized staple oligonucleotides (e.g., 100 µM).

NOTE: Origami structures usually contain several hundreds of staple strands. Staples are typically purchased from vendors specializing in the chemical synthesis of DNA oligonucleotides in multiwell (e.g., 96-well) plates.

2.2. For 500 µL of 10 nM origami, mix 50 µL of Tris-EDTA (TE, 10x), 100 µL of $MgCl_2$ (100 mM), 25 µL of NaCl (100 mM), 170 µL of H_2O, 100 µL of SM (0.5 µM), 5 µL of lock strands (5 µM), and a 50 µL scaffold (100 nM).

2.3. Anneal the mixture in a thermocycler from 80 °C to 20 °C as described in **Table 1**.

3. DNA origami purification

NOTE: This section describes the protocol for agarose gel purification. DNA origami templates can also be purified using alternative approaches[38,39].

3.1. For 1% gel, dissolve 1 g of agarose in 100 mL of Tris-borate-EDTA (TBE, 0.5x) by heating the mixture in a microwave oven. Add 10,000 µL of 10x DNA stain according to the stain specification. To minimize the exposure to UV light at the extraction step (step 3.6), use a DNA stain that can be visualized under blue excitation.

3.2. Cool the solution to approximately 40 °C and slowly add 1 mL of $MgCl_2$ (1.3 M) while shaking. Cast gel and incubate for 30 min at room temperature.

3.3. Set the electrophoresis device and pour cold (4 °C) running buffer (0.5x TBE with 11 mM $MgCl_2$) into the gel box. Place the gel box in an ice water bath.

3.4. Add loading buffer to the origami samples (6x loading buffer contains 15% polysucrose 400 and 0.25% bromophenol blue in water). Load the samples into the wells with a proper volume according to the comb used (e.g., 50 µL for an 8-well comb of 1.5 mm in thickness).

3.5. Run the electrophoresis for 2 h at 80 V.

NOTE: To characterize the origami and separate the open and closed structure, use 2% gel instead of 1% and prolong the running time to 4 h.

3.6. Image the gel with the gel imager (**Figure 2**). Use a blue light transilluminator to visualize the bands, cut the origami band, smash the gel on a parafilm, and extract the liquid. The recovery yield is approximately 40%.

3.7. Pipette the liquid into a centrifugal filter unit and spin at 3,000 x *g* for 5 min. Measure the absorption of the origami solution at 260 nm with a UV-visible (UV-VIS) spectrometer. Estimate the concentration of origami using an extinction coefficient of 1.3×10^8 $M^{-1} \cdot cm^{-1}$.

NOTE: The typical concentration of origami solution after agarose gel purification is 1–2 nM.

3.8. Store the purified origami templates at 4 °C for later use.

4. Synthesis of gold nanorods

NOTE: The protocol for AuNR synthesis is adapted from previous literature[40] with minor modifications.

4.1. Wash all glassware with aqua regia for 5 min, rinse it with water, sonicate it with ultrapure water, and dry it before use.

4.2. Prepare 0.2 M hexadecyltrimethylammonium bromide (CTAB), 1 mM $HAuCl_4$, 4 mM $AgNO_3$, 64 mM L(+)-ascorbic acid, and 6 mM $NaBH_4$. Use cold water (4 °C) to dissolve $NaBH_4$ and keep it in a fridge at 4 °C. Ascorbic acid solution has to be freshly prepared.

CAUTION: CTAB is hazardous in case of skin contact (irritant), eye contact (irritant), ingestion, and inhalation. Wear suitable protective clothing. In case of insufficient ventilation, wear suitable respiratory equipment. $NaBH_4$ is extremely hazardous in case of skin contact (irritant), eye contact (irritant), ingestion, and inhalation. Wear splash goggles, a lab coat, gloves, and a vapor and dust respirator. Be sure to use an approved/certified respirator or equivalent.

4.3. Prepare Au seeds.

4.3.1. Add 500 µL of CTAB (0.2 M), 250 µL of ultrapure water, and 250 µL of $HAuCl_4$ (1 mM) into a glass vial. Stir at 450 rpm at room temperature for 5 min.

4.3.2. Increase the stirring rate to 1,200 rpm. Add 100 µL of cold $NaBH_4$ solution (6 mM, 4 °C). After 2 min, stop the stirring and incubate the solution in a water bath at 30 °C for 30 min before use.

4.4. Prepare AuNRs.

4.4.1. Dissolve 0.55 g of CTAB and 0.037 g of 2,6-dihydroxybenzoic acid in 15 mL of warm water (60–65 °C) in a round-bottom flask. Cool down the solution to 30 °C, add 600 µL of $AgNO_3$ (4 mM), and stir at 450 rpm for 2 min. Then, leave the solution undisturbed for 15 min at 30 °C.

4.4.2. Add 15 mL of $HAuCl_4$ (1 mM) to the solution, and stir at 450 rpm for 15 min. Add 120 µL of L(+)-ascorbic acid (64 mM), and then, immediately, stir at 1,200 rpm for 30 s. Add 12 µL of Au seeds, and keep stirring at 1,200 rpm for 30 s.

4.4.3. Incubate the solution in a water bath at 30 °C for 18 h. Do not disturb the solution and use a cap to close the flask.

4.4.4. Transfer the resultant solution to centrifuge tubes, and centrifuge at 9,500 x *g* for 12 min at 20 °C. Discard the supernatant, disperse the pellet in 20 mL of ultrapure water, and perform one more centrifugation step.

4.4.5. Disperse the final pellet in 3 mL of distilled water. Estimate the concentration of AuNRs from a UV-VIS absorption measurement using the extinction coefficient for the longitudinal plasmon resonance. The extinction coefficient can be predicted using AuNR shape parameters[41]. Store the AuNRs at 4 °C for further use.

5. Functionalization of gold nanorods with single-stranded DNA

NOTE: This section describes the protocol for AuNR functionalization with single-stranded DNA (ssDNA), following the so-called low pH route adapted from previous literature[42]. The AuNRs covered with DNA are purified by centrifugation; alternatively, the purification can be performed using agarose gel electrophoresis.

5.1. Incubate 20 µL of thiol-functionalized polyT DNA strands (1 mM) with 20 µL of freshly prepared tris(2-carboxyethyl)phosphine hydrochloride (TCEP, 14 mM) for 1 h to reduce disulfide bonds.

NOTE: The thiol groups form bonds with AuNRs, and the polyT sequence hybridizes with the $polyA_{10}$ handle on the origami, in which too many or too few base pairs may lead to a malfunction or an unstable assembly.

CAUTION: TCEP can cause severe skin burns and eye damage. Wear protective gloves/protective clothing/eye protection/face protection.

5.2. Mix 150 µL of AuNRs (10 nM) and 40 µL of TCEP-treated thiol-DNA (0.5 mM). Add 1% sodium dodecyl sulfate (SDS) to the AuNR solution to reach a final SDS concentration of 0.05%. Adjust the pH to 2.5–3 with ~1 µL of HCl (1 M).

5.3. Add 40 µL of TCEP-treated thiol-DNA (0.5 mM) to the AuNR solution. Incubate for 2 h while shaking at 70 rpm.

NOTE: The AuNR-to-DNA ratio should be in the order of 1:5,000–15,000, depending on the size of the rods. For the AuNRs (70 x 30 nm) prepared following the protocol described in section 4, a 13,000 excess of thiol-DNA is recommended.

5.4. Add NaCl to reach a final NaCl concertation of 0.5 M and incubate for 4 h at room temperature while shaking at 70 rpm.

NOTE: A color change at this step may indicate a failed DNA functionalization.

5.5. Adjust the pH to ~8.5 with TBE buffer (10x) and incubate overnight.

5.6. Wash the DNA-AuNRs 4x by mixing the samples with 1,000 mL of washing buffer (0.5x TBE with 0.1% SDS), and centrifuge at 7,000 x *g* for 30 min. Remove the supernatant and resuspend the DNA-AuNRs in the remaining liquid (~40 µL). Estimate the concentration of DNA-AuNRs from a UV-VIS absorption measurement as described in step 4.4.5.

NOTE: The solution might become slightly 'cloudy' at steps 5.3–5.4 due to the CTAB replacement from the surface of the AuNRs by thiol-DNA. The solution should become clear upon warming up to ~35 °C for 5 min.

6. Assembly of gold nanorods on DNA origami templates

6.1. Add $MgCl_2$ to the solution of purified DNA-AuNRs, to a final concentration of 10 mM. Mix the purified DNA-AuNRs and origami to a 10:1 ratio.

NOTE: A lower ratio may decrease the product yield[43].

6.2. Anneal the mixture in a mixer with a temperature control from 40 °C to 20 °C while shaking at 400 rpm, following the procedure in **Table 2**.

NOTE: For CD characterization, the sample can be measured after this step without further purification.

6.3. Use 0.7% agarose gel electrophoresis (3.5 h at 80 V) to purify the final origami-AuNR structures.

6.4. Use a white light transilluminator for imaging. Cut the product band (origami-AuNR dimer) (**Figure 3**), smash the gel on a parafilm, and extract the liquid. Pipette the liquid into a centrifugal filter unit and spin at 3,000 x *g* for 5 min. Resuspend the origami-AuNRs in the solution. The recovery yield from the gel is approximately 50%.

6.5. Estimate the concentration of the origami-AuNR structures from a UV-VIS absorption measurement as described in step 4.4.5.

7. Transmission electron microscopy imaging

NOTE: This uranyl formate (UFo) staining protocol is adapted from previous literature[44].

7.1. Mix 200 µL of UFo solution (0.75%) and 1 µL of NaOH (5 M) and vortex immediately for 2–3 min. Centrifuge the stain solution for 3–4 min at 14,000 x *g*. Protect the stain from light exposure (e.g., by wrapping it in aluminum foil).

CAUTION: UFo is toxic if inhaled or swallowed and can cause eye irritation. In the case of brief exposure or low pollution, use a respiratory filter device. In the case of intensive or longer exposure, use a self-contained respiratory protective device. Wear gloves. The glove material has to be impermeable and resistant to UFo and its solutions. Wear tightly sealed goggles.

7.2. Glow-discharge carbon/formvar-coated TEM grids for 6 s just before use to increase hydrophilicity and promote the sticking of the structures. Pipette 5 µL sample drops on the TEM grid, incubate for 5–8 min, and remove the drop by gently touching a filter paper with the edge of the grid.

7.3. Pipette one big (~20 µL) and one small (~10 µL) drop of the stain solution on a parafilm. Put the grid on the small stain solution drop and dry immediately by touching the filter paper with the edge of the grid. Then, put it on the big stain solution drop for 30 s.

7.4. Remove the liquid on the grid by touching the filter paper with the edge of the grid. Place the grid in the grid holder. Wait for the grid to dry for at least 10 min.

7.5. Characterize the samples of origami (**Figure 4**), AuNRs (**Figure 5**), and origami-AuNRs (**Figure 6**) by TEM.

8. Circular dichroism measurement

8.1. Purge the CD spectrometer with N_2 for 20 min.

NOTE: Most of the CD spectrometers require purging with N_2 before lamp ignition. Check the CD spectrometer manual.

8.2. Set the bandwidth, scanning range, and acquisition step.

NOTE: The scanning range depends on the optical properties of AuNRs, which depend on the size of the AuNRs.

8.3. Measure blank CD with buffer.

8.4. Measure the CD spectra of origami-AuNR samples (**Figure 7**).

NOTE: Use quarts or glass cuvettes for CD measurement. Plastic cuvettes are unsuitable for CD spectroscopy. Also, most CD spectrometers allow the simultaneous acquisition of absorption and CD data.

REPRESENTATIVE RESULTS:

TEM images of DNA origami templates, AuNRs, and final origami-AuNR assemblies are shown in **Figure 4**, **Figure 5**, and **Figure 6A**, respectively. Due to their binding preference to TEM grids, origami-AuNR assemblies are usually seen as parallel origami bundles and rods (**Figure 6A**). Thermal annealing is required for the correct alignment of AuNRs on origami templates (**Figure 6A,B**). The protocol enables high yields of the assembly of AuNRs into chiral metamolecules with strong plasmonic CD responses (**Figure 7**).

FIGURE AND TABLE LEGENDS:

Table 1: Temperatures and rates for the thermal annealing of DNA origami templates.

Table 2: Temperatures and holding times for the annealing of AuNRs and DNA origami templates. The cooling rate between the steps is set at 0.1 °C/min. The DNA origami-AuNR samples are annealed while shaking at 400 rpm.

Figure 1: Design of DNA origami-templated chiral metamolecules. (**A**) Identify the desired relative spatial arrangement of gold nanorods (AuNRs) and a suitable shape of the DNA origami template. (**B**) Estimate the structural parameters of the AuNRs (D_{AuNR}, L_{AuNR}) and the origami template ($W_{origami}$, $L_{origami}$, Θ). Locate the approximate positions of the staples that need further modification. (**C**) Design of DNA origami templates using caDNAno.

Figure 2: The agarose gel electrophoresis of origami. (**A**) Purification with 1% agarose gel electrophoresis for 2 h at 80 V. (**B**) Characterization with 2% agarose gel electrophoresis for 4 h at 80 V.

Figure 3: The agarose gel electrophoresis purification of origami-AuNRs. Gel (0.5%) was run for 3.5 h at 80 V for the samples prepared following the assembly procedure with different DNA-AuNR-to-origami ratios (20:1, 5:1) and samples (10:1 DNA-AuNRs-to-origami ratio) with/without annealing procedure. For TEM images of the samples in bands 1, 2, and 3, see **Figure 6**.

Figure 4: Representative TEM image of the DNA origami templates. The origami structure consists of two 14-helix bundles (80 nm x 16 nm x 8 nm) linked together by the scaffold strand.

Figure 5: Representative TEM image of the AuNRs. The average dimensions of synthesized AuNRs are 70 x 30 nm.

Figure 6: TEM images of origami-AuNR assemblies. (**A**) AuNR dimers on origami after annealing (band 1 in **Figure 3**). (**B**) AuNR dimers on origami without annealing (band 2 in **Figure 3**). (**C**) Origami-AuNR aggregates (band 3 in **Figure 3**).

Figure 7: CD spectra of the origami-AuNR assemblies. The CD spectra of the closed structures (the origami templates fixed by lock strands into a right-handed configuration, with 50° between two origami bundles) and the open structure (the origami templates without lock strands).

DISCUSSION:

The protocol introduces the whole workflow of design, assembly, purification, and characterization of DNA origami-based chiral assemblies of AuNRs. The DNA origami templates used in the protocol are particularly suitable for the fabrication of stimuli-responsive assemblies. Various types of responses and functionalizes can be incorporated into the lock strands that define the chiral state of the origami template (**Figure 1B**)[24–26,31]. For static assemblies, simpler block-shaped templates are often sufficient[14,45–47].

The DNA origami-based approach to the fabrication of plasmonic nanostructure inherits limitations of the DNA origami technique[48]. The size of the origami templates is typically limited by the size of the scaffold strand. The stability of DNA structures is reduced under law-salt conditions. The cost of synthetic stable strands remains rather high. However, recent developments in the field of structural DNA nanotechnology are expected to overcome these limitations[49–55].

Compared to other molecular-based approaches for generating chiral assemblies of AuNRs[34–37], DNA origami provides a high level of spatial precision and programmability.

For achieving reliable and reproducible optical responses of chiral assemblies, we strongly recommend adapting the protocols for AuNR synthesis[40], since the quality and optical properties of commercial products may vary between batches. Additional annealing (step 6.2) is often crucial for ensuring the correct attachment of AuNRs to DNA origami templates (**Figure 6**).

Finally, the protocol described here is not limited to chiral assemblies. DNA origami provides a very flexible platform for the fabrication of complex plasmonic nanostructures[9,10].

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DISCLOSURES:

The authors have nothing to disclose.

REFERENCES

1. Rothemund, P. W. K. Folding DNA to create nanoscale shapes and patterns. *Nature.* **440**, 297–302 (2006).
2. Wang, P., Meyer, T. A., Pan, V., Dutta, P. K., Ke, Y. The Beauty and Utility of DNA Origami. *Chem.*

2, 359–382 (2017).

3. Hong, F., Zhang, F., Liu, Y., Yan, H. DNA Origami: Scaffolds for Creating Higher Order Structures. *Chemical Reviews.* **117**, 12584–12640 (2017).
4. Roller, E.-M., Argyropoulos, C., Högele, A., Liedl, T., Pilo-Pais, M. Plasmon–Exciton Coupling Using DNA Templates. *Nano Letters.* **16**, 5962–5966 (2016).
5. Acuna, G. P. et al. Fluorescence Enhancement at Docking Sites of DNA-Directed Self-Assembled Nanoantennas. *Science.* **338**, 506–510 (2012).
6. Roller, E.-M. et al. DNA-Assembled Nanoparticle Rings Exhibit Electric and Magnetic Resonances at Visible Frequencies. *Nano Letters.* **15**, 1368–1373 (2015).
7. Liu, Q., Song, C., Wang, Z.-G., Li, N., Ding, B. Precise organization of metal nanoparticles on DNA origami template. *Methods.* **67**, 205–214 (2014).
8. Roller, E.-M. et al. Hotspot-mediated non-dissipative and ultrafast plasmon passage. *Nature Physics.* **13**, 761–765 (2017).
9. Liu, N., Liedl, T. DNA-Assembled Advanced Plasmonic Architectures. *Chemical Reviews.* **118**, 3032–3053 (2018).
10. Kuzyk, A., Jungmann, R., Acuna, G. P., Liu, N. DNA Origami Route for Nanophotonics. *ACS Photonics.* **5**, 1151–1163 (2018).
11. Kuzyk, A. et al. DNA-based self-assembly of chiral plasmonic nanostructures with tailored optical response. *Nature.* **483**, 311–314 (2012).
12. Shen, X. et al. Three-Dimensional Plasmonic Chiral Tetramers Assembled by DNA Origami. *Nano Letters.* **13**, 2128–2133 (2013).
13. Liu, H., Shen, X., Wang, Z.-G., Kuzyk, A., Ding, B. Helical nanostructures based on DNA self-assembly. *Nanoscale.* **6**, 9331–9338 (2014).
14. Shen, X. et al. 3D plasmonic chiral colloids. *Nanoscale.* **6**, 2077–2081 (2014).
15. Urban, M. J. et al. Plasmonic Toroidal Metamolecules Assembled by DNA Origami. *Journal of the American Chemical Society.* **138**, 5495–5498 (2016).
16. Hentschel, M., Schäferling, M., Duan, X., Giessen, H., Liu, N. Chiral plasmonics. *Science Advances.* **3**, e1602735 (2017).
17. Cecconello, A., Besteiro, L. V., Govorov, A. O., Willner, I. Chiroplasmonic DNA-based nanostructures. *Nature Reviews Materials.* **2**, 17039 (2017).
18. Lan, X., Wang, Q. Self-Assembly of Chiral Plasmonic Nanostructures. *Advanced Materials.* **28**, 10499–10507 (2016).
19. Shen, C. et al. Spiral Patterning of Au Nanoparticles on Au Nanorod Surface to Form Chiral AuNR@AuNP Helical Superstructures Templated by DNA Origami. *Advanced Materials.* **29**, 1606533 (2017).
20. Zhou, C., Duan, X., Liu, N. A plasmonic nanorod that walks on DNA origami. *Nature Communications.* **6**, 8102 (2015).
21. Douglas, S. M. et al. Rapid prototyping of 3D DNA-origami shapes with caDNAno. *Nucleic Acids Research.* **37**, 5001–5006 (2009).
22. Kim, D.-N., Kilchherr, F., Dietz, H., Bathe, M. Quantitative prediction of 3D solution shape and flexibility of nucleic acid nanostructures. *Nucleic Acids Research.* **40**, 2862–2868 (2012).
23. Maffeo, C., Yoo, J., Aksimentiev, A. De novo reconstruction of DNA origami structures through atomistic molecular dynamics simulation. *Nucleic Acids Research.* **44**, 3013–3019 (2016).
24. Kuzyk, A. et al. Reconfigurable 3D plasmonic metamolecules. *Nature Materials.* **13**, 862–866

(2014).
25. Kuzyk, A. et al. A light-driven three-dimensional plasmonic nanosystem that translates molecular motion into reversible chiroptical function. *Nature Communications.* **7**, 10591 (2016).
26. Kuzyk, A., Urban, M. J., Idili, A., Ricci, F., Liu, N. Selective control of reconfigurable chiral plasmonic metamolecules. *Science Advances.* **3**, e1602803 (2017).
27. Gerling, T., Wagenbauer, K. F., Neuner, A. M., Dietz, H. Dynamic DNA devices and assemblies formed by shape-complementary, non–base pairing 3D components. *Science.* **347**, 1446–1452 (2015).
28. Zhou, C., Duan, X., Liu, N. DNA-Nanotechnology-Enabled Chiral Plasmonics: From Static to Dynamic. *Accounts of Chemical Research.* **50**, 2906–2914 (2017).
29. Ijäs, H. et al. Dynamic DNA Origami Devices: from Strand-Displacement Reactions to External-Stimuli Responsive Systems. *International Journal of Molecular Sciences.* **19**, 2114 (2018).
30. Lan, X. et al. DNA-Guided Plasmonic Helix with Switchable Chirality. *Journal of the American Chemical Society.* **140**, 11763–11770 (2018).
31. Funck, T., Nicoli, F., Kuzyk, A., Liedl, T. Sensing Picomolar Concentrations of RNA Using Switchable Plasmonic Chirality. *Angewandte Chemie International Edition.* **57**, 13495–13498 (2018).
32. Zhou, C., Xin, L., Duan, X., Urban, M. J., Liu, N. Dynamic Plasmonic System That Responds to Thermal and Aptamer-Target Regulations. *Nano Letters.* **18**, 7395–7399 (2018).
33. Zhan, P. et al. Reconfigurable Three-Dimensional Gold Nanorod Plasmonic Nanostructures Organized on DNA Origami Tripod. *ACS Nano.* **11**, 1172–1179 (2017).
34. Ma, W. et al. Attomolar DNA detection with chiral nanorod assemblies. *Nature Communications.* **4**, 2689 (2013).
35. Ma, W. et al. Chiral plasmonics of self-assembled nanorod dimers. *Scientific Reports.* **3**, 1934 (2013).
36. Guerrero-Martínez, A. et al. Intense Optical Activity from Three-Dimensional Chiral Ordering of Plasmonic Nanoantennas. *Angewandte Chemie International Edition.* **50**, 5499–5503 (2011).
37. Kumar, J. et al. Detection of amyloid fibrils in Parkinson's disease using plasmonic chirality. *Proceedings of the National Academy of Sciences.* **115**, 3225–3230 (2018).
38. Stahl, E., Martin, T. G., Praetorius, F., Dietz, H. Facile and Scalable Preparation of Pure and Dense DNA Origami Solutions. *Angewandte Chemie International Edition.* **53**, 12735–12740 (2014).
39. Shaw, A., Benson, E., Högberg, B. Purification of Functionalized DNA Origami Nanostructures. *ACS Nano.* **9**, 4968–4975 (2015).
40. Ye, X. et al. Improved Size-Tunable Synthesis of Monodisperse Gold Nanorods through the Use of Aromatic Additives. *ACS Nano.* **6**, 2804–2817 (2012).
41. Near, R. D., Hayden, S. C., Hunter, R. E., Thackston, D., El-Sayed, M. A. Rapid and Efficient Prediction of Optical Extinction Coefficients for Gold Nanospheres and Gold Nanorods. *The Journal of Physical Chemistry C.* **117**, 23950–23955 (2013).
42. Shi, D., Song, C., Jiang, Q., Wang, Z.-G., Ding, B. A facile and efficient method to modify gold nanorods with thiolated DNA at a low pH value. *Chemical Communications.* **49**, 2533–2535 (2013).
43. Gür, F. N., Schwarz, F. W., Ye, J., Diez, S., Schmidt, T. L. Toward Self-Assembled Plasmonic Devices: High-Yield Arrangement of Gold Nanoparticles on DNA Origami Templates. *ACS Nano.*

10, 5374–5382 (2016).

44. Castro, C. E. et al. A primer to scaffolded DNA origami. *Nature Methods.* **8**, 221–229 (2011).

45. Lan, X. et al. Bifacial DNA Origami-Directed Discrete, Three-Dimensional, Anisotropic Plasmonic Nanoarchitectures with Tailored Optical Chirality. *Journal of the American Chemical Society.* **135**, 11441–11444 (2013).

46. Lan, X. et al. Au Nanorod Helical Superstructures with Designed Chirality. *Journal of the American Chemical Society.* **137**, 457–462 (2015).

47. Zhu, C., Wang, M., Dong, J., Zhou, C., Wang, Q. Modular Assembly of Plasmonic Nanoparticles Assisted by DNA Origami. *Langmuir*. doi:10.1021/acs.langmuir.8b01933 (2018).

48. Pinheiro, A. V., Han, D., Shih, W. M., Yan, H. Challenges and opportunities for structural DNA nanotechnology. *Nature Nanotechnology.* **6**, 763–772 (2011).

49. Ducani, C., Kaul, C., Moche, M., Shih, W. M., Högberg, B. Enzymatic production of ‘monoclonal stoichiometric’ single-stranded DNA oligonucleotides. *Nature Methods.* **10**, 647–652 (2013).

50. Praetorius, F. et al. Biotechnological mass production of DNA origami. *Nature.* **552**, 84–87 (2017).

51. Wagenbauer, K. F., Sigl, C., Dietz, H. Gigadalton-scale shape-programmable DNA assemblies. *Nature.* **552**, 78–83 (2017).

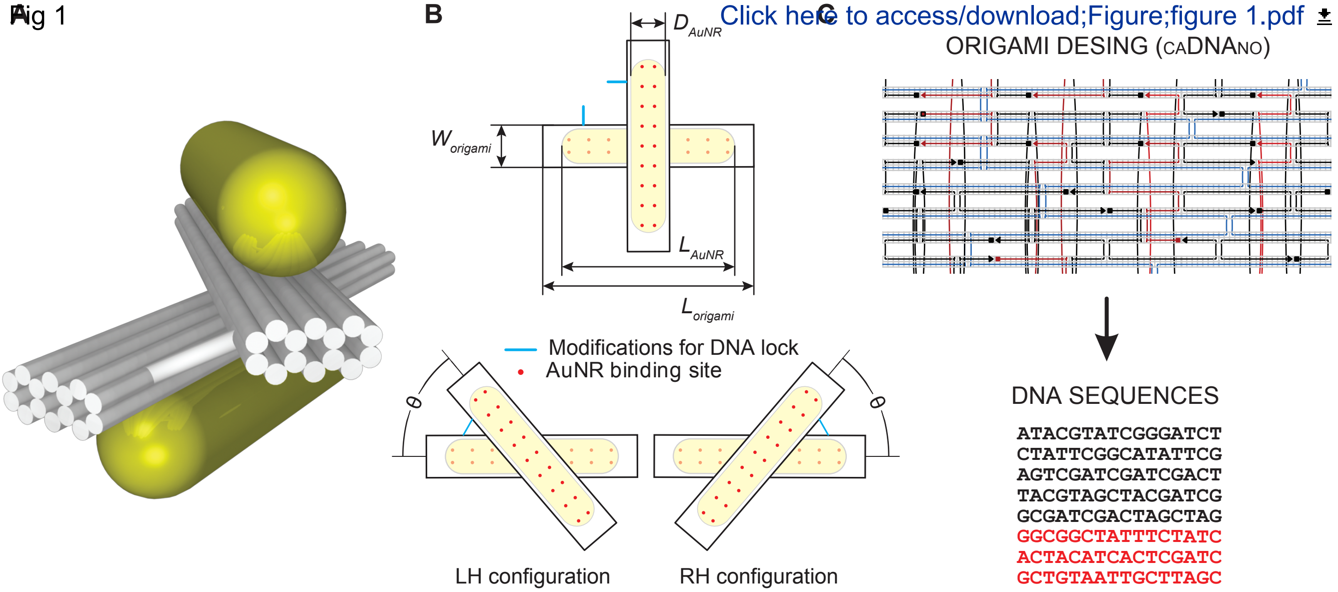
52. Zhang, T. et al. 3D DNA Origami Crystals. *Advanced Materials.* **30**, 1800273 (2018).

53. Ong, L. L. et al. Programmable self-assembly of three-dimensional nanostructures from 10,000 unique components. *Nature.* **552**, 72–77 (2017).

54. Ponnuswamy, N. et al. Oligolysine-based coating protects DNA nanostructures from low-salt denaturation and nuclease degradation. *Nature Communications.* **8**, 15654 (2017).

55. Agarwal, N. P., Matthies, M., Gür, F. N., Osada, K., Schmidt, T. L. Block Copolymer Micellization as a Protection Strategy for DNA Origami. *Angewandte Chemie International Edition.* **56**, 5460–5464 (2017).

Fig 1


A
B
C
D_{AuNR}
$W_{origami}$
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Modifications for DNA lock
AuNR binding site
θ
θ
LH configuration
RH configuration
ORIGAMI DESING (caDNAno)
DNA SEQUENCES
ATACGTATCGGGATCT
CTATTCGGCATATTCG
AGTCGATCGATCGACT
TACGTAGCTACGATCG
GCGATCGACTAGCTAG
GGCGGCTATTTCTATC
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Fig 2

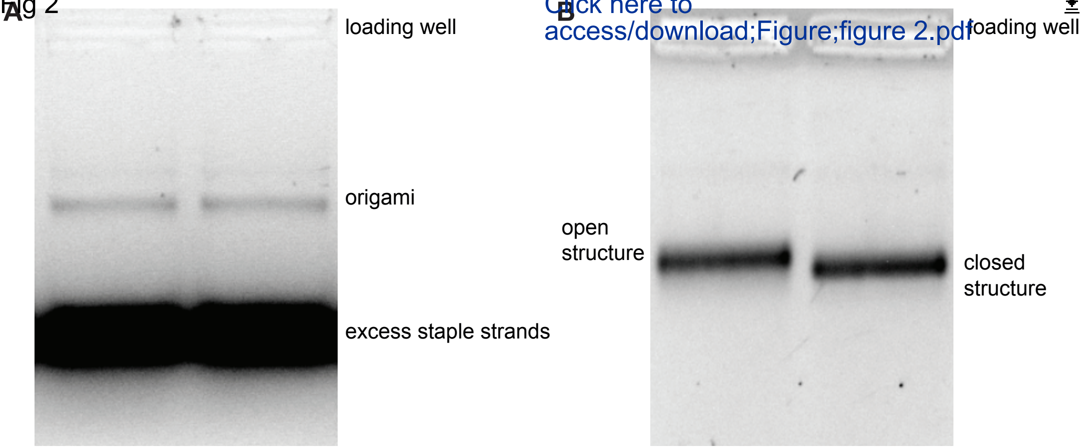

A
loading well
origami
excess staple strands
B
loading well
open structure
closed structure

Fig 3

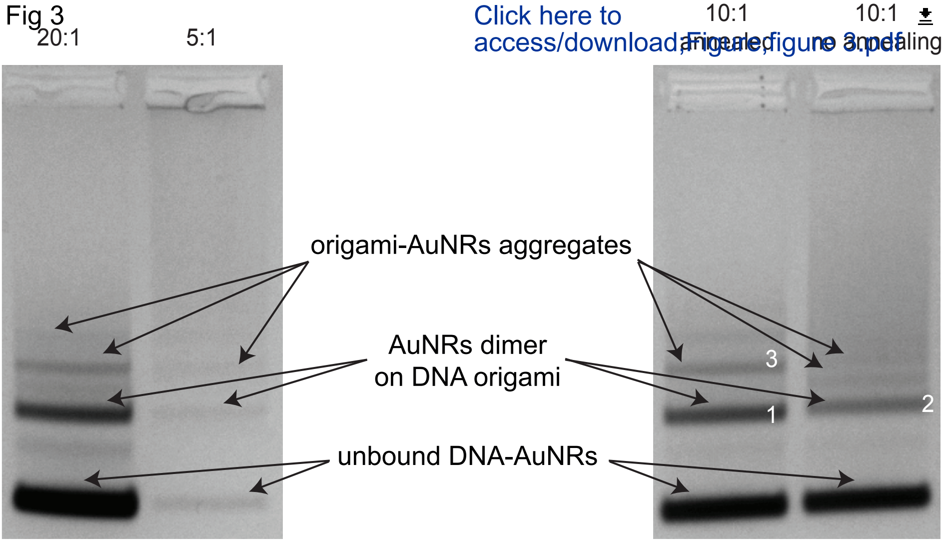

20:1
5:1
10:1
annealing
10:1
no annealing
origami-AuNRs aggregates
AuNRs dimer
on DNA origami
unbound DNA-AuNRs
3
1
2

Fig 4

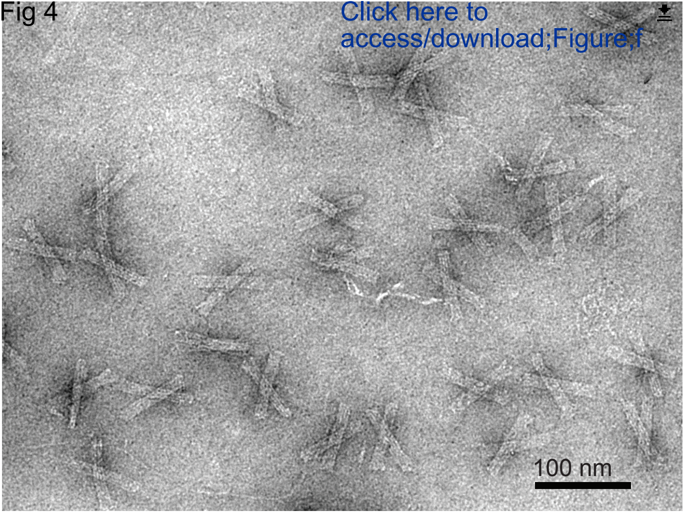


Fig 5

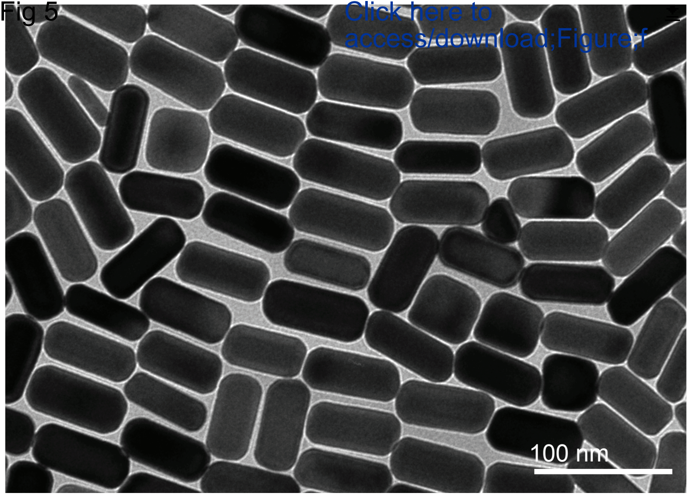


Fig 6

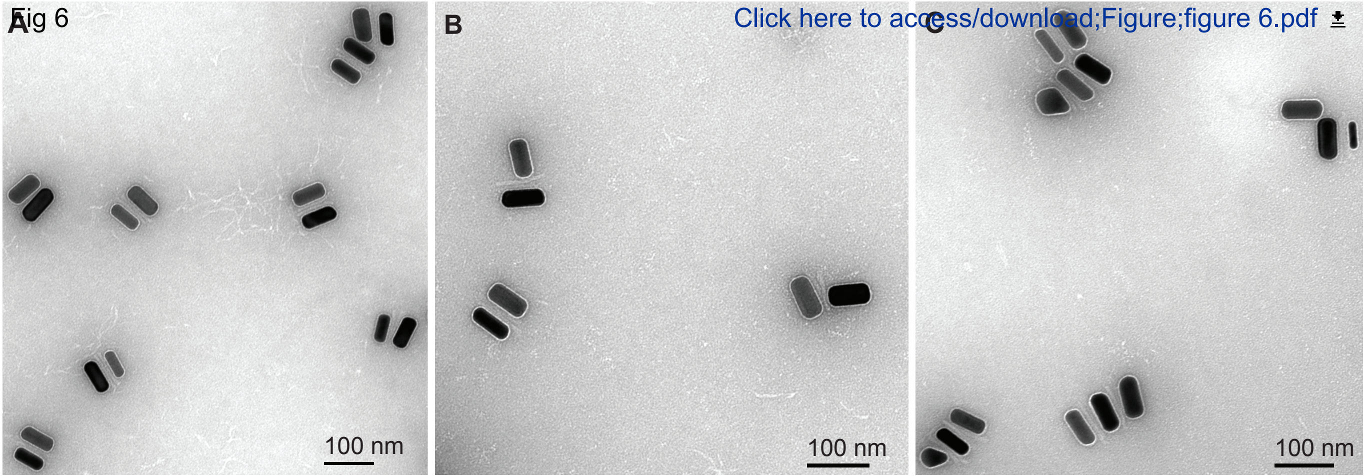
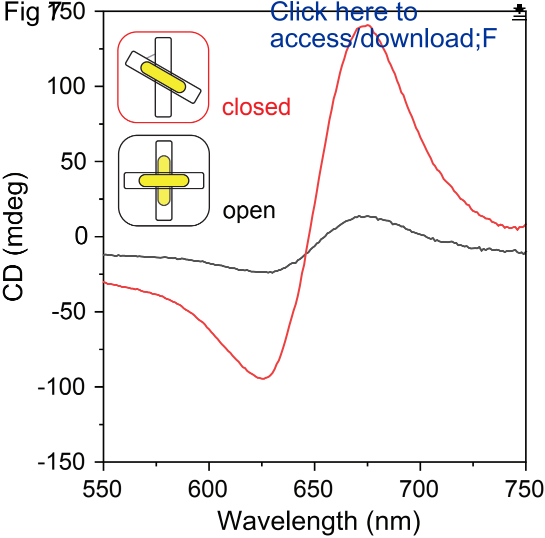


Fig 7



150
100
50
0
-50
-100
-150
CD (mdeg)
closed
open
550
600
650
700
750
Wavelength (nm)

NOTE: caDNAno design file

Click here to access/download
Video or Animated Figure
caDNAno desing of origami templates.json

Temperature (°C)	Time
80	15 min
79 - 71	1 °C / 1 min
70 - 66	1 °C / 5 min
65 - 60	1 °C / 30 min
59 - 37	1 °C / 60 min
36 - 30	1 °C / 15 min
29 - 20	1 °C / 5 min
20	Hold

Temperature (°C)	Time (min)
40	130
36	180
32	180
22	Hold

Name	Company	Catalog #	Comments
2,6-Dihydroxybenzoic acid	Sigma-Aldrich	D109606-25	98+%
$AgNO_3$	Alfa Aesar	AA1141414	99.90%
Blue light transilluminator	Nippon Genetics	FG-06	FastGene LED Transilluminator
Bromophenol Blue	Acros Organics	403160050	For agarorose gel loading buffer
Centrifugal filter units	Merck Millipore	42600	DNA extraction from agarose
Chirascan CD spectrometer	Applied Photophysics		
Cuvette	Hellma	105-202-85-40	Quartz SUPRASIL
DNA lobind tubes	Eppendorf	30108051	
Eppendorf Biospectrometer	Eppendorf	6135000904	
Eppendorf ThermoMixer C	Eppendorf	5382000015	
Ficoll 400	Thermo Fisher Scientific	BP525-10	Polysucrose 400 (For agarorose gel loading buffer)
Gel electrophoresis sets	Thermo Fisher Scientific		
Gel imager	Bio-Rad		Gel Doc XR+ System
$HAuCl_4$•$3H_2O$	Alfa Aesar	AA3640006	99.99%
HCl	Scharlau	AC07441000	1M
Hexadecyltrimethylammonium bromide (CTAB)	Sigma-Aldrich	H9151-100	BioXtra, 98+%
L(+)-ascorbic acid	Acros Organics	401471000	99+%
M13p7560 scaffold strand	Tilibit nanosystems		
$MgCl_2$•$6H_2O$	Sigma-Aldrich	M2670-500	BioXtra, 99+%
$NaBH_4$	Acros Organics	200050250	99%
NaCl	Sigma-Aldrich	S7653-500	BioXtra, 99.5+%
NaOH	Sigma-Aldrich	S8045-500	BioXtra, 98+%
Parafilm	Sigma-Aldrich	P7668-1EA	PARAFILM M
PBS buffer (10X)	Thermo Fisher Scientific	BP3991	Molecular Biology
ProFlex PCR System	Thermo Fisher Scientific	4484073	

Sodium dodecyl sulfate (SDS)	Sigma-Aldrich	74255-250	99+%
Staple strands	Thermo Fisher Scientific		
Sybr Safe	Invitrogen	S33102	For DNA stain
TBE buffer (10X)	Invitrogen	15581-044	Molecular Biology
TE buffer (10X)	Thermo Fisher Scientific	BP24771	Molecular Biology
TEM	FEI		FEI Tecnai F12
Thiol-functionalized ssDNA	Biomers.net		
Tris(2-carboxyethyl)phosphine hydrochloride (TCEP-HCl)	Thermo Fisher Scientific	PI20491	
UltraPure Agarose	Invitrogen	16500-100	
Ultrapure water (Type 1)			Milli-Q Direct 8 system
Uranyl Formate	Tebu-bio	24762-1	
White light transilluminator	UVP	TW-26	

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Please find enclosed our revised manusript JoVE59280 *"Assembly of gold nanorods into chiral plasmonic metamolecules using DNA origami templates".*
The manuscript is revised according to editorial comments.
Our responses to comments are below.
We hope the manuscripts is now suitable for publication in JoVE.

With kind regards,
Anton Kuzyk

Editorial comments:

The manuscript has been modified and the updated manuscript, **59280_R1.docx**, is attached and located in your Editorial Manager account. **Please use the updated version to make your revisions.**

1. Please take this opportunity to thoroughly proof read the manuscript to ensure that there are no spelling or grammar issues.

2. For steps that are done using software, a step-wise description of software usage must be included in the step. Please mention what button is clicked on in the software, or which menu items need to be selected to perform the step.

We added step-wise description of software.

3. Please convert centrifuge speeds to centrifugal force (x g) instead of revolutions per minute (rpm) or rcf.
We converted all centrifuge speeds to centrifugal force as requested.

4. Please use h, min, s for time units.
We edited the time units as requested

5. Steps 1.1-1.4: These steps cannot be filmed unless detailed software usage is provided.

We modified the section 1 of the protocol with software usage instructions. Step 1.1 is removed from filming.

6. Step 3.4: What's the composition of loading buffer?
We added a description for the composition of loading buffer.

7. Figure 4: Please provide a short description of the figure in addition to the figure title in Figure Legend.

We added a short description to Figure 4.

8. Figure 5: Please provide a short description of the figure in addition to the figure title in Figure Legend.

We added a short description to Figure 5.

9. Please revise the Discussion to explicitly cover the following in detail in 3-6 paragraphs with citations:
a) Critical steps within the protocol
b) Any modifications and troubleshooting of the technique
c) Any limitations of the technique

We added description of limitation of the techniques.

Note: the critical steps are already covered by the paragraph

*For achieving reliable and reproducible optical responses of chiral assemblies, we strongly recommend adapting the protocols for AuNRs synthesis[40], since the quality and optical properties of commercial products may vary between batches. Additional annealing (step 6.2) is often crucial for ensuring the correct attachment of AuNRs to DNA origami templates (**Figure 6**).*

We really do not have anything to add for points b).

TITLE:

Assembly of gold nanorods into chiral plasmonic metamolecules using DNA origami templates

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KEYWORDS:

DNA nanotechnology, gold nanorods, DNA origami, self-assembly, chiral plasmonics, circular dichroism.

SUMMARY:

We describe the detailed protocol for DNA origami-based assembly of gold nanorods into chiral plasmonic metamolecules with strong chiroptical responses. The protocol is not limited to chiral configurations and can be easily adapted for fabrication of various plasmonic architectures.

ABSTRACT:

Inherent addressability of the DNA origami structures makes them ideal templates for arrangement of metal nanoparticles into complex plasmonic nanostructures. High spatial precision of DNA origami templated assembly allows controlling the coupling between plasmonic resonances of individual particles and enables tailoring optical properties of the constructed nanostructures. Recently, chiral plasmonic systems attracted a lot of attention due to the strong correlation between the spatial configuration of plasmonic assemblies and their optical responses, e.g., circular dichroism. In this protocol, we describe the whole workflow for generation of DNA origami-based chiral assemblies of gold nanorods (AuNRs). The protocol includes detailed description of design principles and experimental procedures for fabrication DNA origami templates, synthesis of AuNRs, and assembly of origami-AuNRs structures. In addition, characterization of structures using transmission electron microscopy (TEM) and circular dichroism (CD) spectroscopy is included. The described protocol is not limited to chiral configurations and can be adapted for the construction of various plasmonic architectures.

INTRODUCTION:

DNA nanostructures, in particular DNA origami, have been widely used to arrange molecules and other nanoscale components, e.g., proteins and nanoparticles (NPs), with nanometer precision into almost arbitrary geometries[1–5]. The ability to arrange metal NPs on DNA origami templates with high yield and accuracy enables the fabrication of plasmonic structures with novel optical properties[6–10]. DNA origami technique is especially useful for the generation of chiral plasmonic structures, which require genuinely three-dimensional architectures[11–20].

This protocol describes in detail the entire process of the fabrication of DNA origami templated chiral assemblies of gold nanorods (AuNRs). The software used for design[21] and structure prediction[22,23] of DNA origami is intuitive and freely available. The origami fabrication and AuNRs synthesis uses common biochemistry lab equipment, e.g, thermo~~s~~cyclers, gel electrophoresis, hot plates, centrifuges, etc. The structures are characterized using standard transmission electron microscopy (TEM) and circular dichroism (CD) spectroscopy.

Fabrication of similar plasmonic nanostructures with top-down methods, e.g., electron beam lithography, would require rather complicated and expensive equipment. In addition, DNA origami templates provide possibility to incorporate structural reconfigurability in plasmonic assemblies[24–33], which is extremely challenging for structures fabricated with lithography techniques. Compared to other molecular based approaches[34–37] DNA origami-based fabrication provides high level of spatial precision and programmability.

PROTOCOL:

1. Design the DNA origami

1.1. Identify desired relative spatial arrangement of AuNRs and suitable shape of DNA origami template (**Figure 1A**). Estimate structural parameters of the AuNRs and the origami templates. Locate approximate positions of staples that need further modification (**Figure 1B**).

1.2. Download and install caDNAno[18] to design DNA origami template. In caDNAno, route the scaffold strand according to the desired shape of the template and generate the staple strands (Click **Seq Tool**). Click **Paint Tool** and m~~M~~ark the staple strands that require further modification, i.e., (**Figure 1C**).

1.3. Click **Export Tool** to export the DNA staple sequences (**Figure 1C**) to csv file. ~~Add polyA$_{10}$ sequence at the end of the staples used for AuNRs assembly (handles).~~

~~Note: assemblies in the representative results contain 36 handles protruding at 3′ end of the staple strands, 18 on each DNA origami bundle equally distributed on 2 parallel helices every 21 nt. The distance between the first and the last handle position is 168 nt, approximately 57 nm (see the attached caDNAno file).~~

1.4. Design double-stranded locks to fix the angle Θ between the two origami bundles.

Depending on the relative orientation of the two bundles, the origami construct can adapt left- or right-handed (LH/RH) chiral spatial configuration (**Figure 1B**). ~~Modify the staple strands on the designed lock sites with lock sequences.~~

1.5. Import the staples csv file in a spreadsheet application. Add polyA_{10} sequence at the end of the staples used for AuNRs assembly (handles). Modify the staple strands on the designed lock sites with lock sequences.

Note: assemblies in the representative results contain 36 handles protruding at 3’ end of the staple strands, 18 on each DNA origami bundle equally distributed on 2 parallel helices every 21 nt. The distance between the first and the last handle position is 168 nt, approximately 57 nm (see the attached caDNAno file).

2. Assembly of the DNA origami templates

2.1. Prepare working stock of staple stands (SM), including strands with handles and locks, by mixing equal amounts of concentration-normalized staple oligonucleotides, e.g.*,* 100 µM).

Note: origami structures usually contain several hundreds of staple strands. Staples are typically purchased from vendors specializing in chemical synthesis of DNA oligonucleotides in multiwell, e.g., 96-well-plates.

2.2. For 500 µL of 10 nM origami, mix 50 µL TE (10X), 100 µL $MgCl_2$ (100 mM), 25 µL NaCl (100 mM), 170 µL H_2O, 100 µL SM (0.5 µM), 5 µL lock strands (5 µM) and 50 µL scaffold (100 nM).

2.3. Anneal the mixture in a thermocycler from 80 °C to 20 °C through process in **Table 1**.

3. DNA origami purification

Note: this section describes protocol for agarose gel purification. DNA origami templates can also be purified using alternative approaches[38,39].

3.1. For 1% gel, dissolve 1 g agarose in 100 mL TBE (0.5X) by heating the mixture in a microwave oven. Add 10000 µL of 10X DNA stain according to the stain specification. To minimize the exposure to UV light at extraction step (step 3.6), use DNA stain that can be visualized under blue excitation.

3.2. Cool the solution to approximately 40 °C and slowly add 1 mL of $MgCl_2$ (1.3 M) with shaking. Cast gel and incubate for 30 min at room temperature.

3.3. Set the electrophoresis devise and pour cold (4 °C) running buffer (0.5X TBE with 11 mM $MgCl_2$) in the gel box. Place the gel box in an ice water bath.

3.4. Add loading buffer to the origami samples (6X loading buffer contains: 15% polysucrose 400 and 0.25% bromophenol blue in water). Load the samples into the wells with proper volume according to the comb used, e.g., 50 µL for an 8-well comb of 1.5 mm thickness.

3.5. Run the electrophoresis for 2 h at 80 V.

Note: to characterize the origami and separate the open and closed structure, use 2% gel instead of 1% and prolong the running time to 4 h.

3.6. Image the gel with the gel imager (**Figure 2**). Use blue light transilluminator to visualize the bands, cut the origami band, smash the gel on a parafilm and extract the liquid. The recovery yield is approximately 40%.

3.7. Pipette the liquid into a centrifugal filter unit and spin at 3000 ~~rcf~~ x g for 5 min. Measure the absorption of the origami solution at 260 nm with a UV-VIS spectrometer. Estimate the concentration of origami using extinction coefficient of 1.3×10^{8} M^{-1} cm^{-1}.

Note: typical concentration of origami solution after agarose gel purification is 1 - 2 nM.

3.8. Store the purified origami templates at 4 °C for later use.

4. Synthesis of gold nanorods

Note: the protocol for AuNRs synthesis is adapted from previous literature[40] with minor modifications.

4.1. Wash all glassware with aqua regia for 5 min, rinse with water, sonicate with ultrapure water, and dry before use.

4.2. Prepare 0.2 M hexadecyltrimethylammonium bromide (CTAB), 1 mM $HAuCl_4$, 4 mM $AgNO_3$, 64 mM L(+)-ascorbic acid, and 6 mM $NaBH_4$. Use cold water (4 °C) to dissolve $NaBH_4$, and keep it in fridge at 4 °C. Ascorbic acid solution has to be freshly prepared.

CAUTION: CTAB is hazardous in case of skin contact (irritant), of eye contact (irritant), of ingestion, of inhalation. Wear suitable protective clothing. In case of insufficient ventilation, wear suitable respiratory equipment.

CAUTION: $NaBH_4$ is extremely hazardous in case of skin contact (irritant), of eye contact (irritant), of ingestion, of inhalation. Wear splash goggles, lab coat, gloves, vapor and dust respirator. Be sure to use an approved/certified respirator or equivalent.

4.3. Prepare Au seeds

4.3.1. Add 500 µL CTAB (0.2 M), 250 µL ultrapure water, and 250 µL $HAuCl_4$ (1 mM) into a glass vial. Stir at 450 rpm at room temperature for 5 min.

4.3.2. Increase the stirring rate to 1200 rpm. Add 100 µL cold $NaBH_4$ solution (6 mM, 4 °C). After 2 min, stop the stirring and incubate the solution in a water bath at 30 °C for 30 min before use.

4.4. Prepare AuNRs

4.4.1. Dissolve 0.55 g CTAB and 0.037 g 2,6-Dihydroxybenzoic acid in 15 mL warm water (60 - 65 °C) into a round bottom flask. Cool down the solution to 30 °C, add 600 µL $AgNO_3$ (4 mM) and stir at 450 rpm for 2 min. Then leave the solution undisturbed for 15 min at 30 °C.

4.4.2. Add 15 mL $HAuCl_4$ (1 mM) to the solution, and stir at 450 rpm for 15 min. Add 120 µL L(+)-Ascorbic acid (64 mM), then immediately, stir at 1200 rpm for 30 s~~ec~~. Add 12 µL Au seeds, and keep stirring at 1200 rpm for 30 s~~ec~~.

4.4.3. Incubate the solution in a water bath at 30 °C for 18 h. Do not disturb, and use a cap to close the flask.

4.4.4. Transfer the resultant solution to centrifuge tubes, and centrifuge at 9500 ~~rcf~~ x g for 12 min at 20°C. Discard the supernatant, disperse the pellet in 20 mL ultrapure water and perform one more centrifugation step.

4.4.5. Disperse the final pellet in 3.0 mL distilled water. Estimate the concentration of AuNRs from UV-VIS absorption measurement using extinction coefficient for the longitudinal plasmon resonance. The extinction coefficient can be predicted using AuNRs shape parameters[41]. Store the AuNRs at 4 °C for further use.

5. Functionalize gold nanorods with single stranded-DNA

Note: this section describes the protocol for AuNRs functionalization with single stranded-DNA (ssDNA) following the so-called low pH route adapted from a previous literature[42]. The AuNRs covered with DNA are purified by centrifugation; alternatively, the purification can be performed using agarose gel electrophoresis.

5.1. Incubate 20 µL thiol-functionalized polyT DNA strands (1 mM) with 20 µL freshly prepared tris(2-carboxyethyl)phosphine hydrochloride (TCEP, 14 mM) for 1 h to reduce disulfide bonds.

Note: the thiol groups form bonds with AuNRs and the polyT sequence hybridize with the $polyA_{10}$ handle on the origami, in which too many or too few base pairs may lead to malfunction or unstable assembly.

CAUTION: TCEP can cause severe skin burns and eye damage. Wear protective gloves/ protective clothing/ eye protection/ face protection.

5.2. Mix 150 µL AuNR (10 nM) and 40 µL TCEP treated thiol-DNA (0.5 mM). Add 1% sodium dodecyl sulfate (SDS) to AuNRs solution to reach a final SDS concentration of 0.05%. Adjust the pH to 2.5 - 3 with ~1 µL HCl (1 M).

5.3. Add 40 µL TCEP treated thiol-DNA (0.5 mM) to AuNRs solution. Incubate for 2 h with shaking at 70 rpm.

Note: the AuNRs to DNA ratio should be in the order of 1:5000-15000 depending on the size of the rods. For the AuNRs (70 x 30 nm) prepared following the protocol described in the section 4, 13000 excess of thiol-DNA is recommended.

5.4. Add NaCl to reach a final NaCl concertation of 0.5 M and incubate for 4 h at room temperature with shaking at 70 rpm.

Note: a color change at this step may indicate a failed DNA functionalization.

5.5. Adjust the pH to ~8.5 with TBE buffer (10X) and incubate overnight.

5.6. Wash the DNA-AuNRs for 4 times by mixing the samples with 1000 mL washing buffer (0.5X TBE with 0.1% SDS) and centrifuge~~ing~~ at 7000 ~~rcf~~ x g for 30 min. Remove the supernatant and resuspend the DNA-AuNRs in the remaining liquid (~40 µL). Estimate the concentration of DNA-AuNRs from UV-VIS absorption measurement as in step 4.4.5.

Note: solution might become slightly 'cloudy' at steps 5.3 - 5.4 due to CTAB replacement from the surface of the AuNRs by thiol-DNA. The solution should become clear upon warming up to ~35 °C for 5 min.

6. Assembly of gold nanorods on DNA origami templates

6.1. Add $MgCl_2$ to the solution of purified DNA-AuNRs to a final concentration of 10 mM. Mix purified DNA-AuNRs and origami with 10:1 ratio.

Note: lower ratio may decrease the product yield[43].

6.2. Anneal the mixture in a mixer with temperature control from 40 °C to 20 °C while shaking at 400 rpm with the procedure in **Table 2**.

Note: for CD characterization, the sample can be measured after this step without further purification.

6.3. Use 0.7% agarose gel electrophoresis (3.5 h at 80 V) to purify the final origami-AuNRs structures.

6.4. Use white light transilluminator for imaging. Cut the product band (origami-AuNRs dimer)

(**Figure 3**), smash the gel on a parafilm and extract the liquid. Pipette the liquid into a centrifugal filter unit and spin at 3000 rcf x g for 5 min. Resuspend the origami-AuNRs in the solution. The recovery yield from the gel is approximately 50%.

6.5. Estimate the concentration of the origami-AuNRs structures from UV-VIS absorption measurement as in step 4.4.5.

7. Transmission electron microscopy imaging

Note: uranyl formate (UFo) staning protocol is adapted from previous literature[44].

7.1. Mix 200 µL UFo solution (0.75%) and 1 µL NaOH (5 M) and vortex immediately for 2 - 3 mintwo to three minutes. Centrifuge the stain solution for 3 - 4 min at 14000 rcfx g. Protect the stain from light exposure, e.g., by wrapping in aluminum foil.

CAUTION: UFo is toxic if inhaled or swallowed and can cause eye irritation. In case of brief exposure or low pollution use respiratory filter device. In case of intensive or longer exposure use self-contained respiratory protective device. Wear gloves. The glove material has to be impermeable and resistant to UFo and its solutions. Wear tightly sealed goggles.

7.2. Glow discharge carbon/formvar coated TEM grids for 6 sec just before use to increase hydrophilicity and promote sticking of the structures. Pipette 5 µL sample drops on the TEM grid, incubate for 5-8 min and remove the drop by gently touching a filter paper with the edge of the grid.

7.3. Pipette one big (~20 µL) and one small (~10 µL) drop of the stain solution on a parafilm. Put the grid on the small stain solution drop and dry immediately by touching the filter paper with the edge of the grid. Then put it on the big stain solution drop for 30 sec.

7.4. Remove the liquid on the grid by touching the filter paper with the edge of the grid. Place the grid in the grid holder. Wait for the grid to dry for at least 10 min.

7.5. Characterize the samples of origami (**Figure 4**), AuNRs (**Figure 5**), and origami-AuNRs (**Figure 6**) by TEM.

8. Circular dichroism measurement

8.1. Purge the CD spectrometer with N_2 for 20 min.

Note: most of the CD spectrometers require purging with N_2 before lamp ignition. Check the CD spectrometer manual.

8.2. Set the bandwidth, scanning range, and acquisition step.

Note: the scanning range depends on the optical properties of AuNRs, which depend on the size of the AuNRs.

8.3. Measure blank CD with buffer.

8.4. Measure the CD spectra of origami-AuNRs samples (**Figure 7**).
Note: i) use quarts or glass cuvettes for CD measurement. Plastic cuvettes are unsuitable for CD spectroscopy. ii) most of the CD spectrometers allow simultaneous acquisition of absorption and CD data.

REPRESENTATIVE RESULTS:
TEM images of DNA origami templates, AuNRs and final origami-AuNRs assemblies are shown in **Figures 4**, **Figures 5** and **Figures 6A** respectively. Due to binding preference to TEM grids origami-AuNRs assemblies are usually seen as parallel origami bundles and rods (**Figure 6A**). Thermal annealing is required for the correct alignment of AuNRs on origami templates (**Figure 6A** and **Figure 6B**). The protocol enables high yields of assembly of AuNRs into chiral metamolecules with strong plasmonic CD responses (**Figure 7**).

FIGURE AND TABLE LEGENDS:
Table 1. Temperatures and rates for thermal annealing of DNA origami templates.

Table 2. Temperatures and holding times for annealing of AuNRs and DNA origami templates. The cooling rate between the steps is set to 0.1 °C/min. The DNA origami- AuNRs samples are annealed while shaking at 400 rpm.

Table 3. Material for all experiments.

Figure 1. Design of DNA origami templated chiral metamolecules. A. Identify desired relative spatial arrangement of gold nanorods (AuNRs) and suitable shape of DNA origami template. **B.** Estimate structural parameters of the AuNRs (D_{AuNR}, L_{AuNR}) and origami template ($W_{origami}$, $L_{origami}$, Θ). Locate approximate positions of staples that need further modification. **C.** Design DNA origami templates using caDNAno.

Figure 2. The agarose gel electrophoresis of origami. A. Purification with 1% agarose gel electrophoresis for 2 h at 80 V. **B**. Characterization with 2% agarose gel electrophoresis for 4 h at 80 V.

Figure 3. The agarose gel electrophoresis purification of origami-AuNRs. 0.5% gel run for 3.5 h at 80 V of samples prepared following the assembly procedure with different DNA-AuNRs to origami ratio (20:1, 5:1) and samples (10:1 DNA-AuNRs to origami ratio) with/without annealing procedure. For TEM images of samples in bands 1, 2, 3 see **Figure 6**.

Figure 4. Representative TEM image of the DNA origami templates. Origami structure consists of two 14-helix bundles (80 nm × 16 nm × 8 nm) linked together by the scaffold strand.

Figure 5. Representative TEM image of the AuNRs. The average dimensions of synthesized AuNRs are 70 x 30 nm.

Figure 6. TEM images of origami-AuNRs assemblies. A. AuNRs dimers on origami after annealing (band 1 in Figure 3). **B.** AuNRs dimers on origami without annealing (band 2 in Figure 3). **C.** Origami-AuNRs aggregates (band 3 in Figure 3).

Figure 7. CD spectra of the origami-AuNRs assemblies. The CD spectra of the closed structures (the origami templates fixed by lock strands into a right-handed configuration with 50° between two origami bundles) and the open structure (the origami templates without lock strands).

DISCUSSION:

The protocol introduces the whole workflow of design, assembly, purification and characterization of DNA origami-based chiral assemblies of AuNRs. DNA origami templates used in the protocol are particularly suitable for the fabrication of stimuli responsive assemblies. Various types of responses and functionalizes can be incorporated into the lock strands that defines the chiral state of the origami template (**Figure 1B**)[24–26,31]. For static assemblies, simpler block-shaped templates are often sufficient[14,45–47].

Compared to other molecular based approaches for generating chiral assemblies of AuNRs[34–37] DNA origami provides high level of spatial precision and programmability.

For achieving reliable and reproducible optical responses of chiral assemblies, we strongly recommend adapting the protocol for AuNRs synthesis[40], since the quality and optical properties of commercial products may vary between batches. Additional annealing (step 6.2) is often crucial for ensuring the correct attachment of AuNRs to DNA origami templates (**Figure 6**).

~~Compared to other molecular based approaches for generating chiral assemblies of AuNRs[34–37] DNA origami provides high level of spatial precision and programmability.~~

The DNA origami-based approach to fabrication of plasmonic nanostructure inherits limitations of DNA origami technique[48]. The size of the origami templates is typically limited by the size of the scaffold strand. The stability of DNA structures is reduced under law salt conditions. The cost of synthetic stable strands remains rather high. However, recent developments in structural DNA nanotechnology are expected to overcome these limitations[49-55].

~~For achieving reliable and reproducible optical responses of chiral assemblies, we strongly recommend adapting~~ the ~~protocols for AuNRs synthesis[40], since the quality and optical properties of commercial products may vary between batches. Additional annealing (step 6.2) is often crucial for ensuring the correct attachment of AuNRs to DNA origami templates (**Figure 6**).~~

Finally, we would like to mention that the protocol described here is not limited to chiral assemblies. DNA origami provides very flexible platform for the fabrication of complex plasmonic nanostructures.

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The authors have nothing to disclose.

REFERENCES
1. Rothemund, P. W. K. Folding DNA to create nanoscale shapes and patterns. *Nature* **440**, 297–302 (2006).
2. Wang, P., Meyer, T. A., Pan, V., Dutta, P. K. & Ke, Y. The Beauty and Utility of DNA Origami. *Chem* **2**, 359–382 (2017).
3. Hong, F., Zhang, F., Liu, Y. & Yan, H. DNA Origami: Scaffolds for Creating Higher Order Structures. *Chemical Reviews* **117**, 12584–12640 (2017).
4. Roller, E.-M., Argyropoulos, C., Högele, A., Liedl, T. & Pilo-Pais, M. Plasmon–Exciton Coupling Using DNA Templates. *Nano Letters* **16**, 5962–5966 (2016).
5. Acuna, G. P. *et al.* Fluorescence Enhancement at Docking Sites of DNA-Directed Self-Assembled Nanoantennas. *Science* **338**, 506–510 (2012).
6. Roller, E.-M. *et al.* DNA-Assembled Nanoparticle Rings Exhibit Electric and Magnetic Resonances at Visible Frequencies. *Nano Letters* **15**, 1368–1373 (2015).
7. Liu, Q., Song, C., Wang, Z.-G., Li, N. & Ding, B. Precise organization of metal nanoparticles on DNA origami template. *Methods* **67**, 205–214 (2014).
8. Roller, E.-M. *et al.* Hotspot-mediated non-dissipative and ultrafast plasmon passage. *Nature Physics* **13**, 761–765 (2017).
9. Liu, N. & Liedl, T. DNA-Assembled Advanced Plasmonic Architectures. *Chemical Reviews* **118**, 3032–3053 (2018).
10. Kuzyk, A., Jungmann, R., Acuna, G. P. & Liu, N. DNA Origami Route for Nanophotonics. *ACS Photonics* **5**, 1151–1163 (2018).
11. Kuzyk, A. *et al.* DNA-based self-assembly of chiral plasmonic nanostructures with tailored optical response. *Nature* **483**, 311–314 (2012).
12. Shen, X. *et al.* Three-Dimensional Plasmonic Chiral Tetramers Assembled by DNA Origami. *Nano Letters* **13**, 2128–2133 (2013).
13. Liu, H., Shen, X., Wang, Z.-G., Kuzyk, A. & Ding, B. Helical nanostructures based on DNA self-assembly. *Nanoscale* **6**, 9331–9338 (2014).
14. Shen, X. *et al.* 3D plasmonic chiral colloids. *Nanoscale* **6**, 2077–2081 (2014).
15. Urban, M. J. *et al.* Plasmonic Toroidal Metamolecules Assembled by DNA Origami. *Journal of the American Chemical Society* **138**, 5495–5498 (2016).
16. Hentschel, M., Schäferling, M., Duan, X., Giessen, H. & Liu, N. Chiral plasmonics. *Science Advances* **3**, e1602735 (2017).

17. Cecconello, A., Besteiro, L. V., Govorov, A. O. & Willner, I. Chiroplasmonic DNA-based nanostructures. *Nature Reviews Materials* **2**, 17039 (2017).
18. Lan, X. & Wang, Q. Self-Assembly of Chiral Plasmonic Nanostructures. *Advanced Materials* **28**, 10499–10507 (2016).
19. Shen, C. *et al.* Spiral Patterning of Au Nanoparticles on Au Nanorod Surface to Form Chiral AuNR@AuNP Helical Superstructures Templated by DNA Origami. *Advanced Materials* **29**, 1606533 (2017).
20. Zhou, C., Duan, X. & Liu, N. A plasmonic nanorod that walks on DNA origami. *Nature Communications* **6**, 8102 (2015).
21. Douglas, S. M. *et al.* Rapid prototyping of 3D DNA-origami shapes with caDNAno. *Nucleic Acids Research* **37**, 5001–5006 (2009).
22. Kim, D.-N., Kilchherr, F., Dietz, H. & Bathe, M. Quantitative prediction of 3D solution shape and flexibility of nucleic acid nanostructures. *Nucleic Acids Research* **40**, 2862–2868 (2012).
23. Maffeo, C., Yoo, J. & Aksimentiev, A. De novo reconstruction of DNA origami structures through atomistic molecular dynamics simulation. *Nucleic Acids Research* **44**, 3013–3019 (2016).
24. Kuzyk, A. *et al.* Reconfigurable 3D plasmonic metamolecules. *Nature Materials* **13**, 862–866 (2014).
25. Kuzyk, A. *et al.* A light-driven three-dimensional plasmonic nanosystem that translates molecular motion into reversible chiroptical function. *Nature Communications* **7**, 10591 (2016).
26. Kuzyk, A., Urban, M. J., Idili, A., Ricci, F. & Liu, N. Selective control of reconfigurable chiral plasmonic metamolecules. *Science Advances* **3**, e1602803 (2017).
27. Gerling, T., Wagenbauer, K. F., Neuner, A. M. & Dietz, H. Dynamic DNA devices and assemblies formed by shape-complementary, non–base pairing 3D components. *Science* **347**, 1446–1452 (2015).
28. Zhou, C., Duan, X. & Liu, N. DNA-Nanotechnology-Enabled Chiral Plasmonics: From Static to Dynamic. *Accounts of Chemical Research* **50**, 2906–2914 (2017).
29. Ijäs, H. *et al.* Dynamic DNA Origami Devices: from Strand-Displacement Reactions to External-Stimuli Responsive Systems. *International Journal of Molecular Sciences* **19**, 2114 (2018).
30. Lan, X. *et al.* DNA-Guided Plasmonic Helix with Switchable Chirality. *Journal of the American Chemical Society* **140**, 11763–11770 (2018).
31. Funck, T., Nicoli, F., Kuzyk, A. & Liedl, T. Sensing Picomolar Concentrations of RNA Using Switchable Plasmonic Chirality. *Angewandte Chemie International Edition* **57**, 13495–13498 (2018).
32. Zhou, C., Xin, L., Duan, X., Urban, M. J. & Liu, N. Dynamic Plasmonic System That Responds to Thermal and Aptamer-Target Regulations. *Nano Letters* **18**, 7395–7399 (2018).
33. Zhan, P. *et al.* Reconfigurable Three-Dimensional Gold Nanorod Plasmonic Nanostructures Organized on DNA Origami Tripod. *ACS Nano* **11**, 1172–1179 (2017).
34. Ma, W. *et al.* Attomolar DNA detection with chiral nanorod assemblies. *Nature Communications* **4**, 2689 (2013).
35. Ma, W. *et al.* Chiral plasmonics of self-assembled nanorod dimers. *Scientific Reports* **3**, 1934 (2013).
36. Guerrero-Martínez, A. *et al.* Intense Optical Activity from Three-Dimensional Chiral Ordering of Plasmonic Nanoantennas. *Angewandte Chemie International Edition* **50**, 5499–5503 (2011).

37. Kumar, J. *et al.* Detection of amyloid fibrils in Parkinson's disease using plasmonic chirality. *Proceedings of the National Academy of Sciences* **115**, 3225–3230 (2018).

38. Stahl, E., Martin, T. G., Praetorius, F. & Dietz, H. Facile and Scalable Preparation of Pure and Dense DNA Origami Solutions. *Angewandte Chemie International Edition ~~50.~~* **53**, 12735–12740 (2014).

39. Shaw, A., Benson, E. & Högberg, B. Purification of Functionalized DNA Origami Nanostructures. *ACS Nano* **9**, 4968–4975 (2015).

40. Ye, X. *et al.* Improved Size-Tunable Synthesis of Monodisperse Gold Nanorods through the Use of Aromatic Additives. *ACS Nano* **6**, 2804–2817 (2012).

41. Near, R. D., Hayden, S. C., Hunter, R. E., Thackston, D. & El-Sayed, M. A. Rapid and Efficient Prediction of Optical Extinction Coefficients for Gold Nanospheres and Gold Nanorods. *The Journal of Physical Chemistry C* **117**, 23950–23955 (2013).

42. Shi, D., Song, C., Jiang, Q., Wang, Z.-G. & Ding, B. A facile and efficient method to modify gold nanorods with thiolated DNA at a low pH value. *Chemical Communications.* **49**, 2533–2535 (2013).

43. Gür, F. N., Schwarz, F. W., Ye, J., Diez, S. & Schmidt, T. L. Toward Self-Assembled Plasmonic Devices: High-Yield Arrangement of Gold Nanoparticles on DNA Origami Templates. *ACS Nano* **10**, 5374–5382 (2016).

44. Castro, C. E. *et al.* A primer to scaffolded DNA origami. *Nature Methods* **8**, 221–229 (2011).

45. Lan, X. *et al.* Bifacial DNA Origami-Directed Discrete, Three-Dimensional, Anisotropic Plasmonic Nanoarchitectures with Tailored Optical Chirality. *Journal of the American Chemical Society.* **135**, 11441–11444 (2013).

46. Lan, X. *et al.* Au Nanorod Helical Superstructures with Designed Chirality. *Journal of the American Chemical Society* **137**, 457–462 (2015).

47. Zhu, C., Wang, M., Dong, J., Zhou, C. & Wang, Q. Modular Assembly of Plasmonic Nanoparticles Assisted by DNA Origami. *Langmuir* (2018). doi:10.1021/acs.langmuir.8b01933

48. Pinheiro, A. V., Han, D., Shih, W. M. & Yan, H. Challenges and opportunities for structural DNA nanotechnology. *Nature Nanotechnology* **6**, 763–772 (2011).

49. Ducani, C., Kaul, C., Moche, M., Shih, W. M. & Högberg, B. Enzymatic production of 'monoclonal stoichiometric' single-stranded DNA oligonucleotides. *Nature Methods* **10**, 647–652 (2013).

50. Praetorius, F. *et al.* Biotechnological mass production of DNA origami. *Nature* **552**, 84–87 (2017).

51. Wagenbauer, K. F., Sigl, C. & Dietz, H. Gigadalton-scale shape-programmable DNA assemblies. *Nature* **552**, 78–83 (2017).

52. Zhang, T. *et al.* 3D DNA Origami Crystals. *Advanced Materials* **30**, 1800273 (2018).

53. Ong, L. L. *et al.* Programmable self-assembly of three-dimensional nanostructures from 10,000 unique components. *Nature* **552**, 72–77 (2017).

54. Ponnuswamy, N. *et al.* Oligolysine-based coating protects DNA nanostructures from low-salt denaturation and nuclease degradation. *Nature Communications* **8**, 15654 (2017).

55. Agarwal, N. P., Matthies, M., Gür, F. N., Osada, K. & Schmidt, T. L. Block Copolymer Micellization as a Protection Strategy for DNA Origami. *Angewandte Chemie International Edition*

56, 5460–5464 (2017).