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Electronic Supplementary Information

A Switchable DNA Origami/Plasmonic Hybrid Device with a Precisely Tuneable DNA-free Interparticle Gap

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Experimental Procedures

1. Materials

All purchased reagents used without further purification.

Table S1. Chemicals/reagents, their specified purity and provider.

Chemical/Reagent	Purity	Company
Agarose	MolBio. grade	Biozym
Ascorbic acid	≥ 99.0 %	AppliChem
Boric acid	99.97 %	Sigma-Aldrich
Cetyltrimethylammonium bromide (CTAB)	≥ 98 %	Sigma-Aldrich
Cetyltrimethylammonium chloride (CTAC)	≥ 98 %	Sigma-Aldrich
Chloroauric acid-trihydrate	≥ 99.9 %	Sigma-Aldrich
Ethidium bromide	MolBio. grade	Merck KGaA
Ethylenediamine tetraacetic acid disodium salt dihydrate	MolBio. grade	VWR Chemicals
Glycerin	≥ 99.5 %	ROTH
HCl	37 %	VWR Chemicals
M13mp18 phage genome derivates (p8064)		Tilibit Nanosystems GmbH
Magnesium chloride hexahydrate	≥ 99 %	Sigma-Aldrich
NaOH	ACS	Sigma-Aldrich
Silver nitrate	≥ 99.99 %	Sigma Aldrich
Sodium bromide	≥ 99.5 %	Sigma-Aldrich
Sodium borohydride	≥ 96.0 %	Sigma-Aldrich
Sodium chloride	≥ 99.6 %	Sigma-Aldrich
Sodium dodecyl sulfate (SDS)	≥ 98.9 %	Sigma-Aldrich
Tris(2-carboxyethyl)phosphine	MolBio. grade	Sigma-Aldrich
Tween20	MolBio. grade	Sigma-Aldrich

2. Buffer solutions

Table S2. Buffer solutions.

Buffer/Solution	Composition
TBE (1x)	89 mM tris base, 89 mM boric acid, 2 mM EDTA, pH 8.0
TBEMg (1x)	40 mM tris base, 20 mM boric acid, 2 mM EDTA, 12.5 mM Mg acetate, pH 8.0
TEMg (1x)	20 mM tris base, 2 mM EDTA, 12.5 mM $MgCl_2$, pH 7.6
1 kb DNA-ladder	Carl Roth GmbH + Co. KG
Gel loading buffer	90 vol. % glycerol, 10 vol. % 10x TEMg 11, bromphenol blue sodium salt

3. Characterisation Devices and Equipment

Table S3. Used equipment.

Equipment	Company
Gel electrophoresis systems	VWR International GmbH
Nanophotometer	DeNovix Inc.
Pipettes: Eppendorf Research Plus	Eppendorf AG
TEM, JEM 1400+	JEOL Ltd.
Thermocyclers	Eppendorf AG
Typhoon FLA 9000	GE Healthcare
UV-Vis, V-730	Jasco
Water purification system	Merck Millipore KGaA
easiGlow	PELCO

4. Syntheses and Assemblies

Synthesis of gold nanospheres.
The synthesis of gold nanospheres (AuNP) was accomplished using a modified protocol published by Park *et al*..[1] Briefly, 10 nm CTAC-capped AuNP were prepared in aqueous solution at 30 °C by first preparing 1-2 nm CTAB-capped gold nanoclusters. Inside a 25 mL glass vial (VWR), 9.75 mL of a 100 mM CTAB solution and 250 µL of a 10 mM $HAuCl_4$ solution were mixed. The resulting yellow solution was stirred at 1,200 rpm after 30 seconds of sonication, followed by a one-shot addition of 600 µL of freshly prepared ice-cold 10 mM $NaBH_4$ solution. After addition, the brownish solution was agitated at 400 rpm for 3 min and incubated at 30 °C for 1 h before use. Then, under gentle stirring at 300 rpm, 10 nm AuNP were produced by mixing 20 mL of a 200 mM CTAC solution with 15 mL of a 100 mM AA solution and 500 mL of freshly prepared gold clusters as described. The resultant reddish colloid was centrifuged twice (20,600 g, 30 min) and redispersed in deionised water for additional functionalisation. The particles were characterised by TEM and UV/Vis absorption spectroscopy (Figure S6).

Functionalisation of gold nanospheres.
To stabilize AuNP under DNA origami buffer conditions (high ionic strength) for further use, a ligand exchange is performed using Tween20 as a stabilising agent. After centrifugation, ligand exchange was carried out by resuspending the particles in 0.5 wt% SDS in two consecutive washing steps. After three additional centrifugation steps, the precipitate was subsequently resuspended in 1% Tween20 solution. The colloid's optical density was adjusted to 1 as monitored via UV-Vis extinction spectroscopy.

DNA origami assembly.
The DNA origami was assembled in a one-pot approach utilising p8064, a derivate of the M13mp18 phage genome, as scaffold. In 1xTEMg 12.5 buffer (20 mM tris base, 2 mM EDTA, 12.5 mM $MgCl_2$), the p8064 scaffold strand (10 nM) was added to a 10-fold excess of each staple strand (100 nM). The thermal annealing technique was carried as the following: heating to 80 °C, temperature hold for 10 min, temperature reduction to 70 °C (2 min/°C), temperature hold for 40 min, temperature reduction to 62 °C (5 min/°C), temperature hold for 2.5 h, temperature reduction to 57 °C (30 min/°C), temperature hold for 4 h, temperature reduction to 45 °C (30 min/°C), temperature hold for 50 min, temperature reduction to 35 °C (5 min/°C), temperature hold for 15 min, temperature reduction to 20 °C (2 min/°C), followed by temperature hold at 20 °C. For further use, DNA origami structures were purified using agarose gel electrophoresis (AGE), band excision and spin-column extraction (Figure S7, S9, S11).

Incorporation of gold nanospheres in Pandora's Boxes.
The incorporation was carried out by deprotection of protected thiols inside Pandora's box (15 nM) in 0.5 mM TCEP (Tris(2-carboxyethyl)phosphine) for 45 min. After incubation, unreacted TCEP was removed using cut-off purification (100 kDa, Amicon Ultra-15). Subsequently, purified origami boxes were mixed with a twentyfold molar excess of Tween-stabilised gold nanospheres in 0.5x TEMg buffer (6.25 mM MgCl, 0.2 wt% Tween20) followed by mixing and further incubation for 24 h at room temperature.

Hybridisation of Pandora's Boxes onto Zelos.
The hybridisation process without incorporated AuNP was performed with a threefold molar excess of purified Pandora (20 nM) concerning purified Zelos (10 nM) at 30 °C with an incubation time of seven days. Hybridisation with incorporated

gold nanospheres was carried out using purified Pandora-AuNP conjugates at lower concentrations (Pandora 0.6 nM, Zelos 0.15 nM) and a fourfold Pandora excess while keeping the incubation time constant. Hybridisation took place in 0.5x TEMg with a $MgCl_2$ concentration of 11 mM to achieve the highest assembly yield possible.

5. DNA origami design

The design of the used origami structures was performed using caDNAno and visualisation of the 3D structures was achieve using Autodesk Maya.

Pandora's Box.
Pandora's Box is designed in a square lattice design, forming a 3D, bilayered origami box. The structure can be divided into four parts, each one consisting of a triangular bottom plate and a rectangular wall. Each bottom plate consists of six double helices, getting shorter towards the inner-most part, with the outermost helix consisting of 88 bp and the inner-most being 11 bp long. Since this may not be sufficient for effective staple binding, the inner scaffold helices are connected by a 56 bases long staple, with 3T spacers between each segment. The walls are 96 bp in lengths and edges are passivated with staples featuring polyT tails at the end in order to minimise dimerisation (dimerisation is still observed to a certain degree). All four parts of the origami are connected by the scaffold (bottom plate) and with staple strands (walls). For incorporation of gold nanoparticles, protruding strands (red) face inside the cavity of the box. Complementary thiolated oligonucleotides (blue) are hybridised to the protruding staples, forming DNA duplexes with thiol groups oriented towards the inner cavity of the box (Figure S1C). Thiolated DNA duplexes can bind gold surfaces and thus initiate incorporation of bare gold nanoparticles. In contrast, base hybridisation approaches, where the gold surface is covered with oligonucleotides and the hot spot is filled with DNA, inhibit functionalization with ligands in the hot spot area.

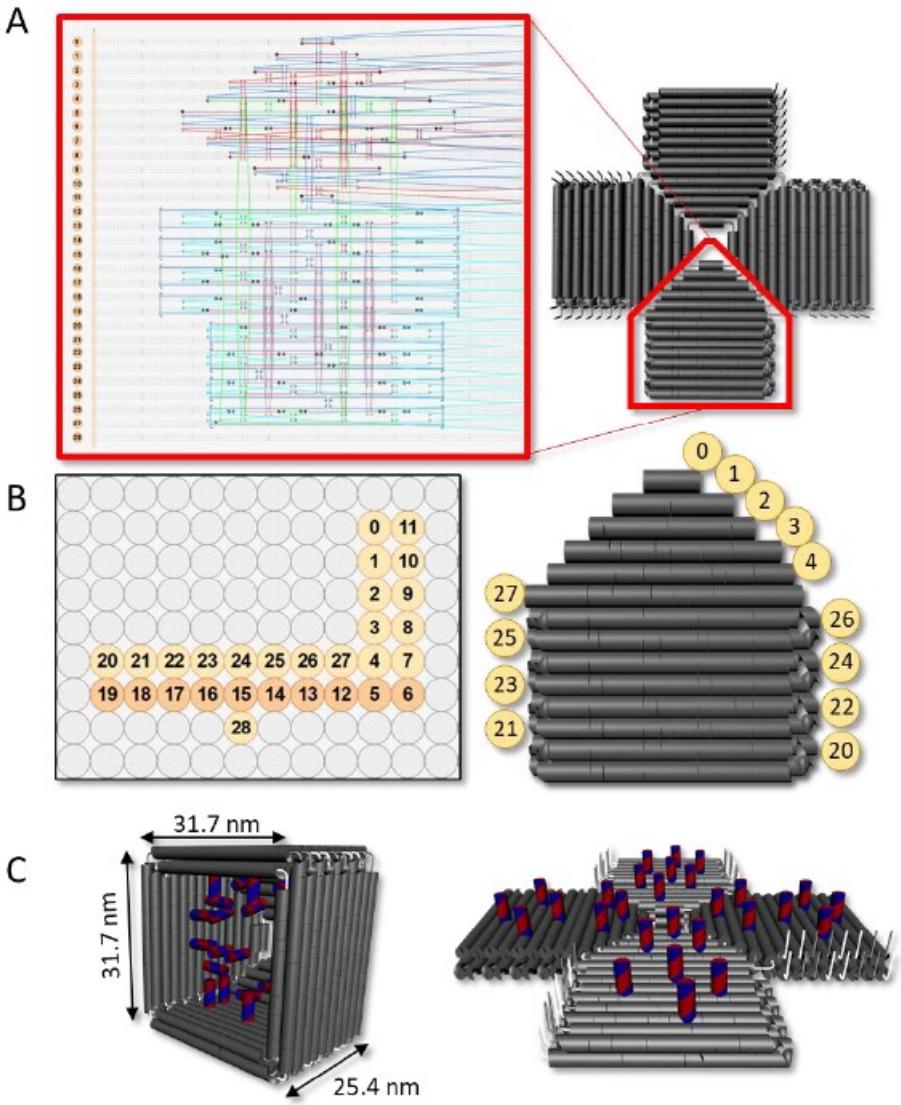
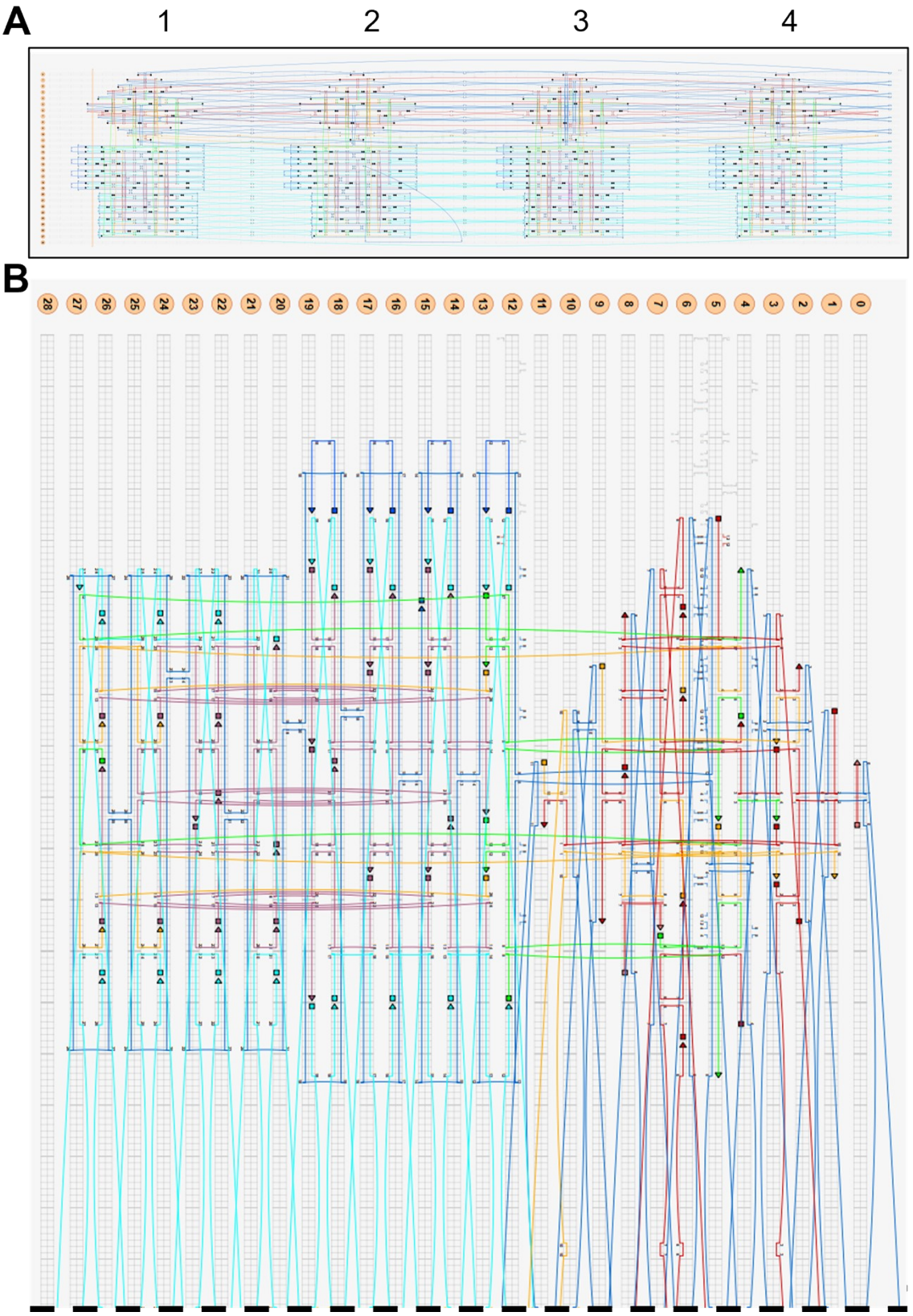


Figure S1. (A) Scaffold and staple pathway of one building block of Pandora's box (illustrated with caDNAno, left) and scheme of Pandora's box design (illustrated with Autodesk Maya, right). (B) Side view of the helical structure (left) and scheme of one building block with corresponding numbering of helices. (C) Scheme of successfully assembled Pandora's box including protruding staples (red) and complementary thiolated oligonucleotides (blue) (left) and unfolded box design (right).[2]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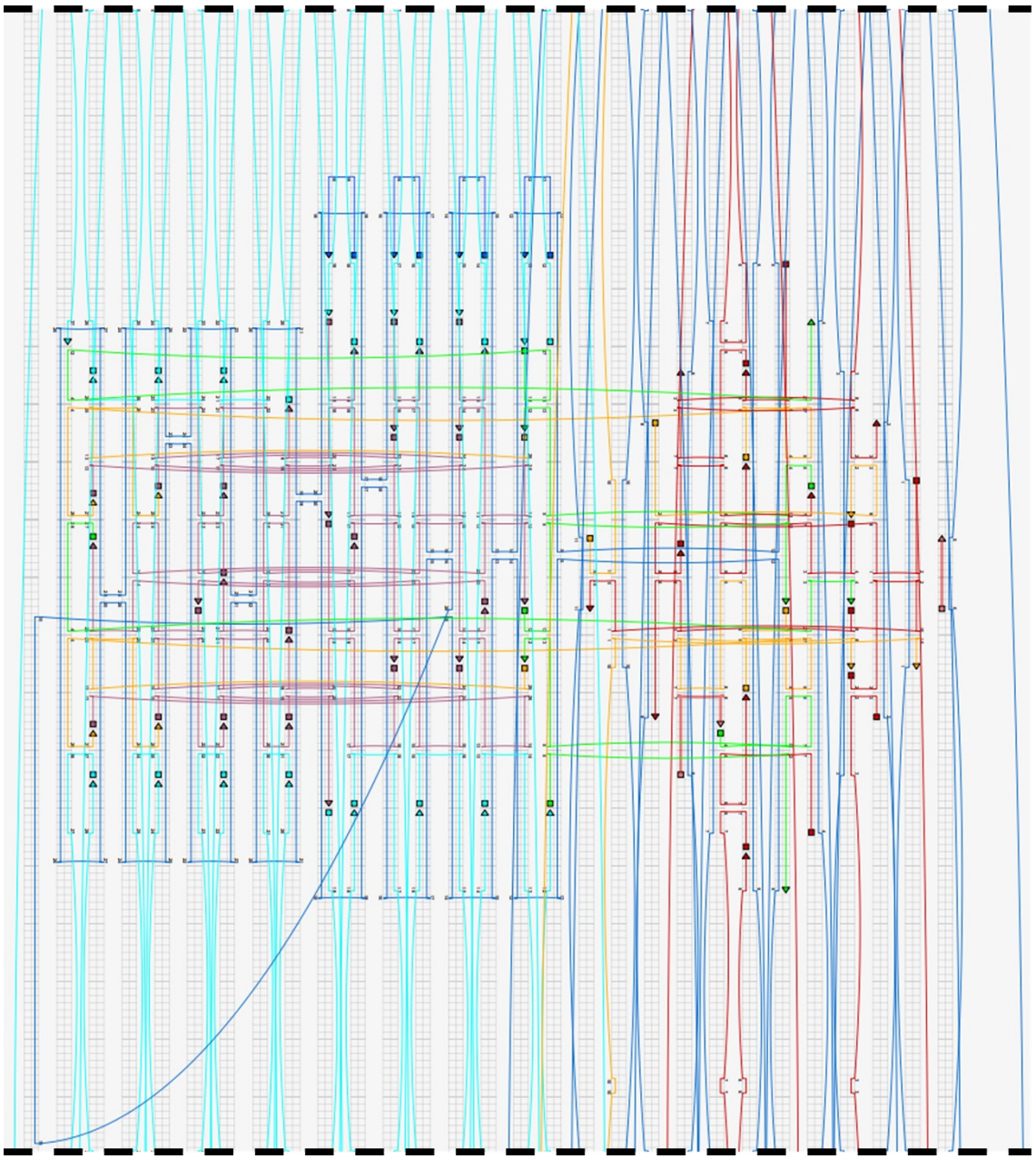

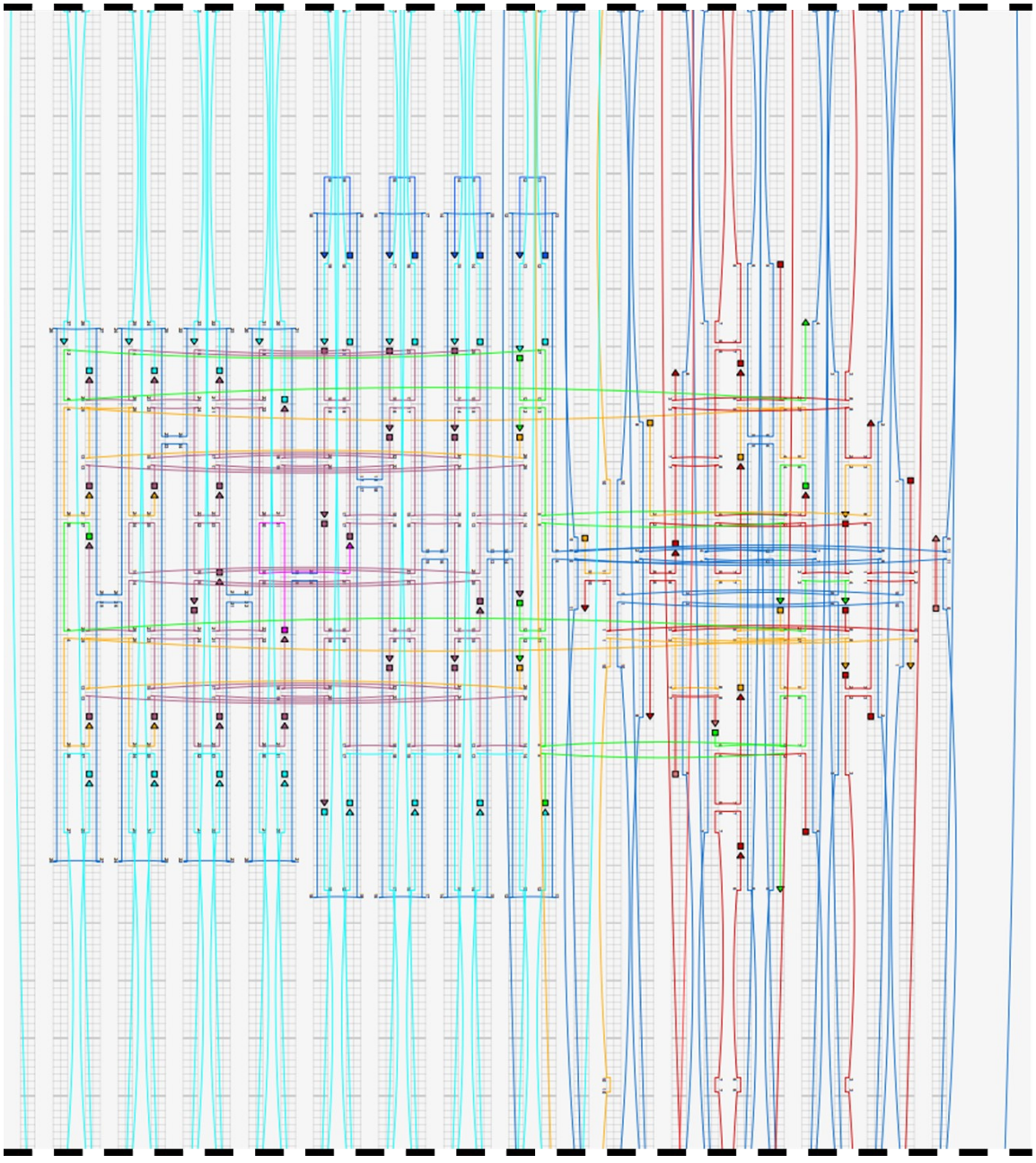

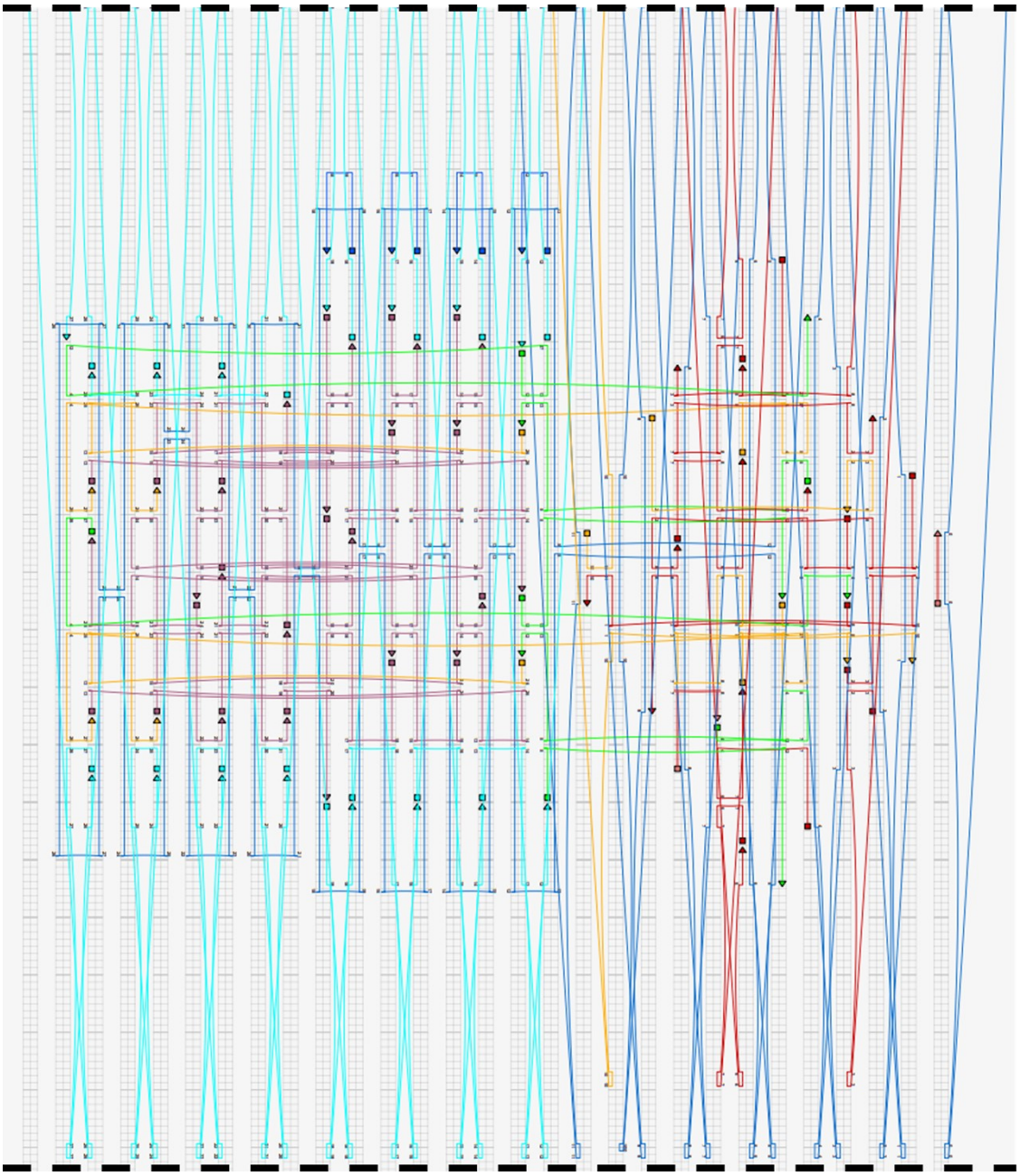

Figure S2: (A) Overview of the caDNAno design of all four sections of Pandora's box. (B) Magnification of the design of each segment; red: structural staples, green: edge passivation staples, purple: wall core staples, cyan: staples connecting walls, dark blue: edge staples, orange: protruding staples.[2]

Zelos.

Zelos was designed as a bilayer origami structure consisting of two rectangles connected by eight helices. These helices enable the dynamic properties of the DNA origami. The bilayer platform consists of 20 parallel helices, each 214 bp long. For hybridisation of Pandora's box onto Zelos, four staples on each rectangle are replaced by protruding staples. Staples at the edges of the structure were modified with poly-T overhangs to prevent unspecific base-stacking between the helices at the opposite sides of the gap and origami-to-origami stacking.

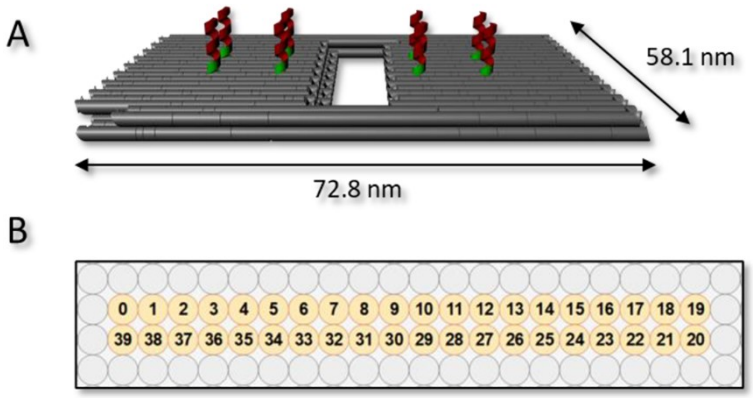

Figure S3: (A) Scheme of Zelos platform with four protruding strands (red) on each rectangular. (B) Side view of the helical structure.[2]

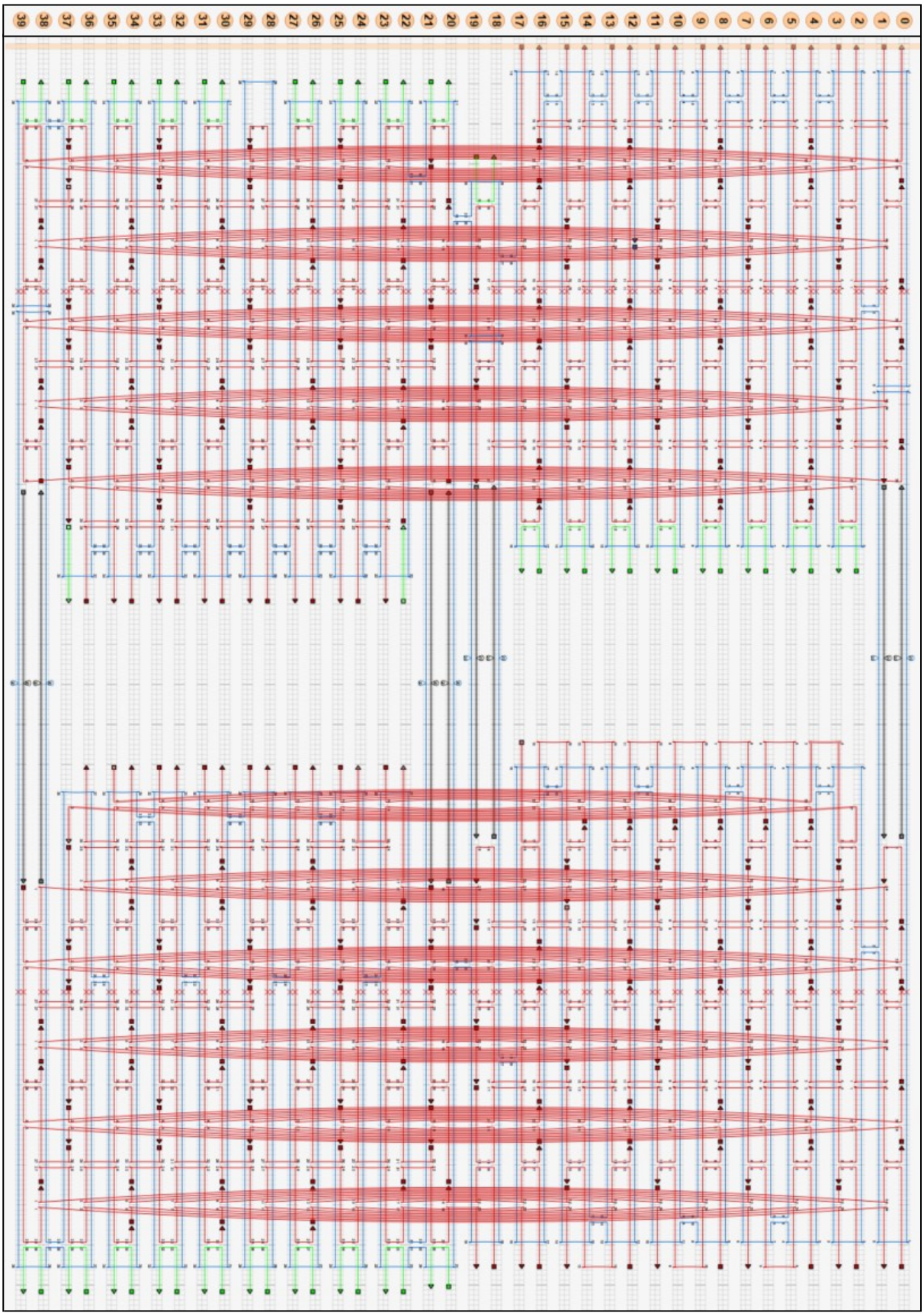

Figure S4: caDNAno design of Zelos platform; red: structural staples, green: edge passivation staples, black: gap staples.[2]

6. Purification Methods

Agarose Gel Electrophoresis.

Agarose Gel Electrophoresis (AGE) was carried out by casting 150 mL of a boiled-up 1 wt% agarose solution in TBEMg 11 buffer in a gel station. After cooling to room temperature, the gel was stored at 4 °C for 1 h prior to use. For gel electrophoresis, the origami samples (10-100 nM) were mixed 1:10 with loading dye buffer and loaded into the gel chambers. For samples including nanoparticles, 0.2 w% Tween20 was included in the gel and running buffer. For analytical gels, 10 µL of 10 nM samples and a 1 kb DNA-ladder as well as scaffold were used during electrophoresis. The electrophoresis was performed for 2 h with a voltage of 80 V for 4 °C using a water-ice bath. After completed AGE, gels were stained using a 1:10,000 ethidiumbromide solution in TBEMg 11 buffer, followed by imaging at a resolution of 25 µM/pixel. Purification of samples was performed by cut-out of gel bands under UV light, enabled through staining or use of fluorophores. Afterwards, commercially available Freeze 'N Squeeze spin columns were used to extract the samples from the gel. The desired bands were frozen inside the tubes at -20 °C for 5 min, followed by centrifugation at 5,000 g at 10 °C for 10 min. Purified origami structures or origami AuNP conjugates were analysed by transmission electron microscopy (TEM).

Transmission Electron Microscopy

TEM was used for characterisation of origami structures, gold nanoparticles and conjugates of these. For visualisation of DNA in TEM, sample staining is required. Therefore, 6 µL of a DNA sample solution with a concentration of 1 nM were applied on a glow discharged (30 s, 15 µA, 25 mbar) carbon film covered copper grid (Quantifoil, copper 400 mesh). After incubation for 60 s, the grid was blotted and 6 µL of a 1 wt% uranyl formate staining solution were added, followed by incubation for 60 s. Afterwards, a second analogue staining step was performed. Finally, the grid was air-dried and analysed by TEM at 120 kV.

Results and Discussion

1. Finite-Difference Time-Domain (FDTD) Simulations

Three dimensional FDTD simulations were performed using the software package from Ansys Lumerical Inc. The computational domain is defined as a 1 µm x 1 µm x 1 µm cubic region. The AuNP dimers are placed at the center of the computational domain. The AuNPs have a diameter of 18 nm and two interparticle gap distances, namely 15 nm and 2 nm are considered. To ensure the accuracy of the simulation and to resolve the electric field inside the gap, a mesh override region is defined around the AuNP dimer with a mesh size of 0.5 nm using the non-uniform conformal variant meshing method. The dielectric permittivity of the gold is modeled using the polynomial fitting of the experimental data obtained by Johnson and Christy.[3] The refractive index of the medium surrounding the AuNP dimer was set to 1.33. The computational domain is illuminated using a broadband (400 - 900 nm) total-field scattered-field (TFSF) source with polarization along the longitudinal axis of the AuNP dimer. Two different 3D analysis groups positioned inside and outside of the excitation source capture the extinction and scattering cross-sections of the AuNP dimer, respectively. Simultaneously, the local electric field profile inside the gap and around the dimers at the resonance wavelength is recorded using three different 2-dimensional frequency-domain field profile monitors positioned in XY, XZ and YZ orientations around the dimers. To avoid any back-reflections into the simulation region and to ensure that all incident electromagnetic fields are completely attenuated, a perfectly matched layer (PML) boundary condition is applied to terminate the computational domain. The total simulation time is set to 200 fs, which is sufficiently long enough for the complete decay of electromagnetic fields in the computational domain.

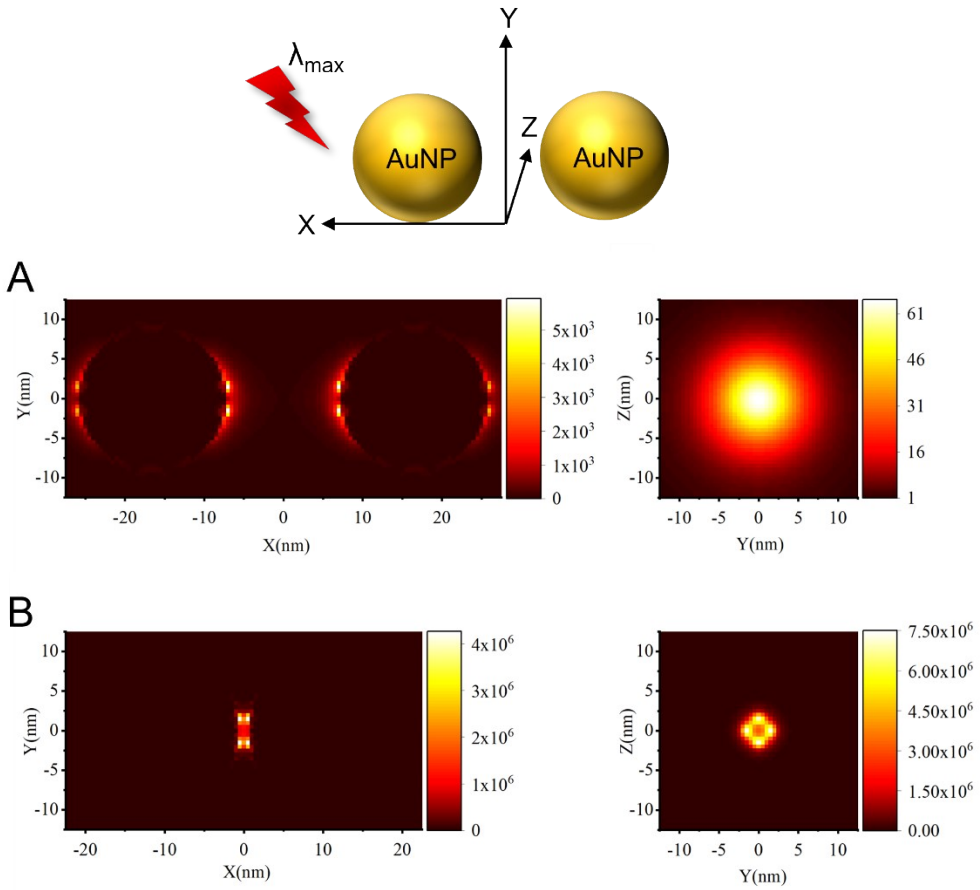

Figure S5. False-color image of SERS enhancement factor distribution (IE^4I) based of FDTD simulations. (A) AuNP dimers with a gap distance of 15 nm. (B) AuNP dimers with a gap distance of 2 nm.[2]

2. Gold Nanospheres

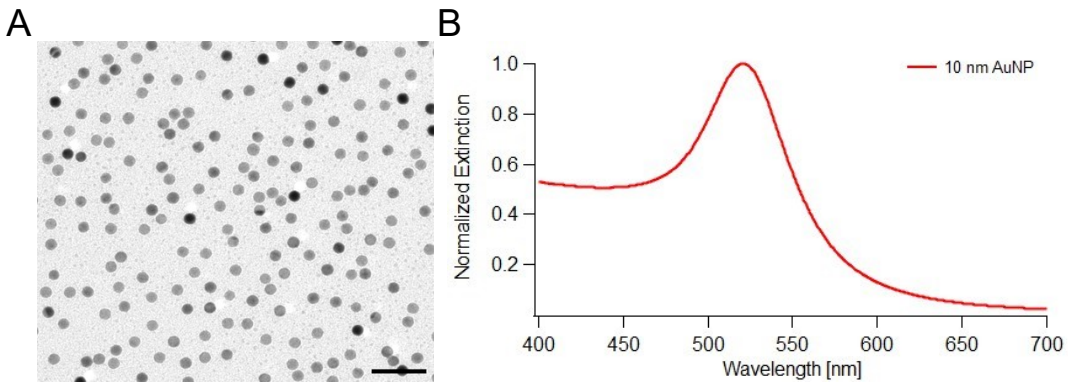

Figure S6. (A) TEM image of 10 nm AuNP. Scale bar: 50 nm. (B) Normalised UV-Vis absorption spectrum of 10 nm AuNP.[2]

3. Pandora's Box

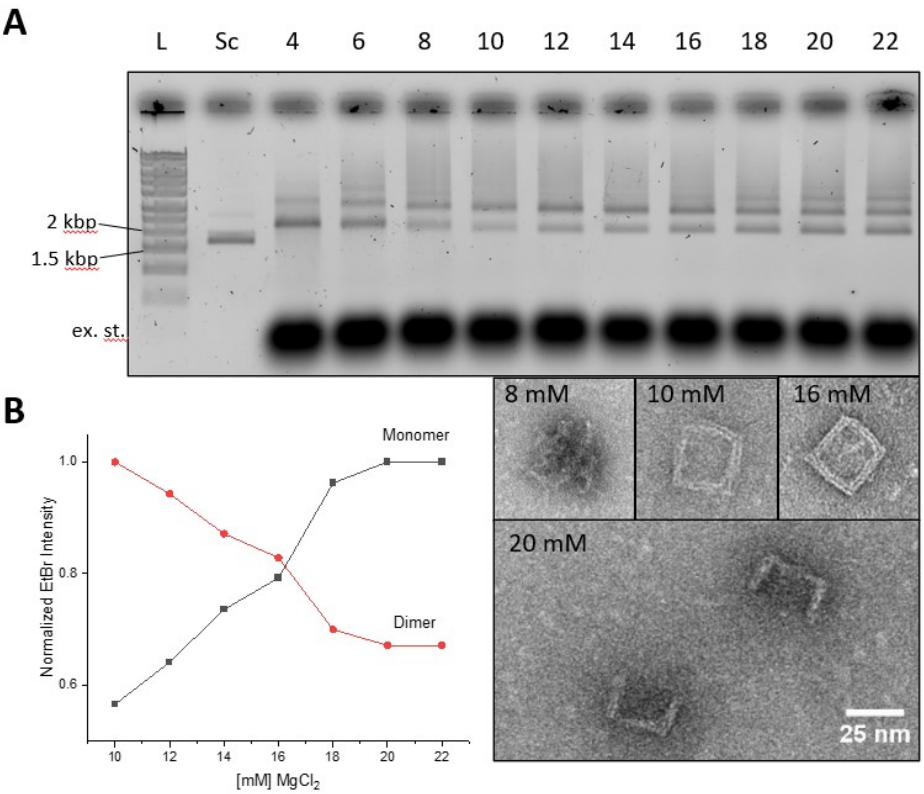

Figure S7. (A) Magnesium-screening of Pandora´s Box assembly; L: 1 kbp ladder, Sc: scaffold, 4-22: $MgCl_2$ concentration used during assembly in mM., ex. st.: excess staple strands. (B) Normalised EtBr fluorescence intensity of the monomer and dimer band, representing the assembly yield (left) and stained TEM images of assembly result with different $MgCl_2$ concentrations (right).[2]

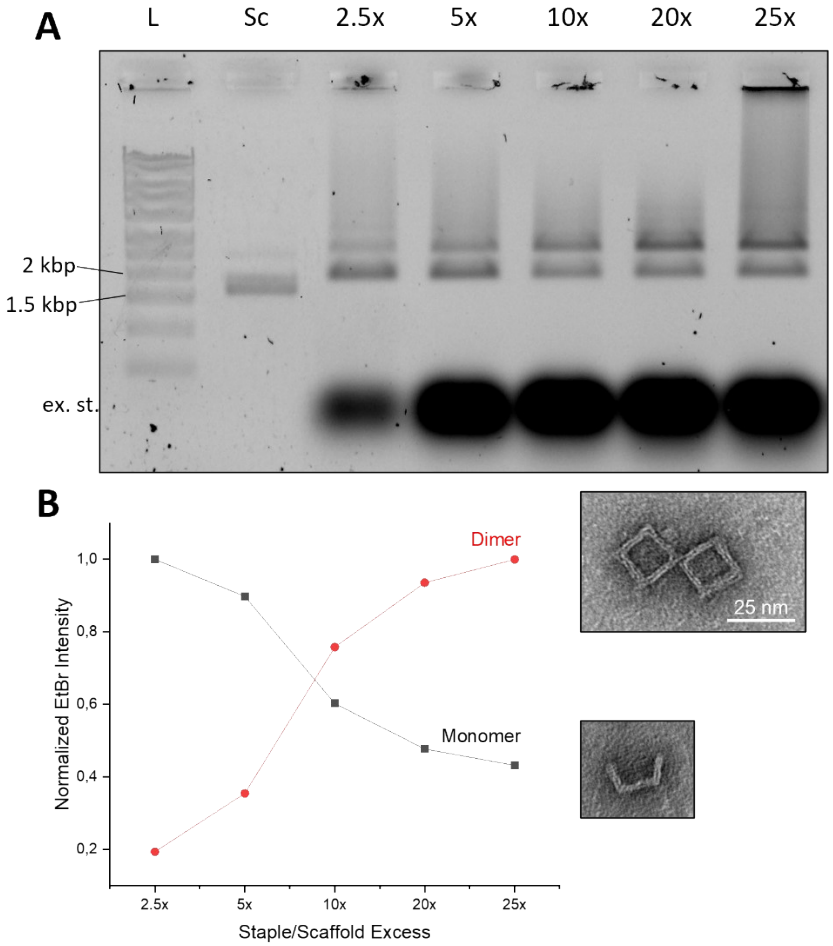

Figure S8. (A) Magnesium-screening of Pandora´s Box assembly; L: 1 kbp ladder, Sc: scaffold, 2.5x – 25x: staple excess used during assembly compared to the scaffold amount, ex. st.: excess staple strands. (B) Normalised EtBr fluorescence intensity of the monomer and dimer band, representing the assembly yield including stained TEM images.[2]

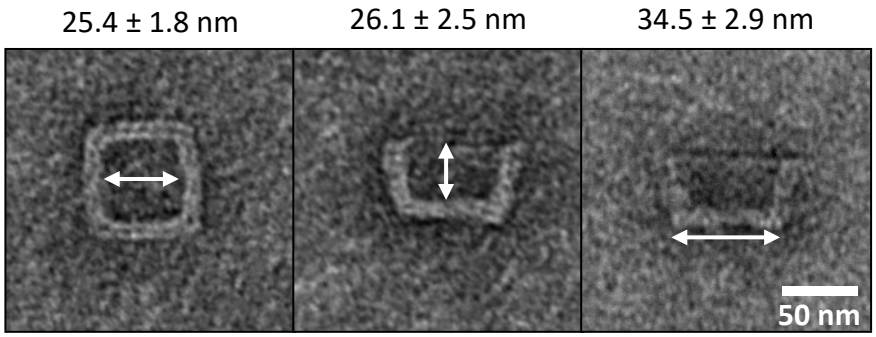

Figure S9. Stained TEM images of Pandora's box for determination of the inner diameter (left), height (middle) and bottom plate (right).

The 3D bilayer DNA origami box consists of four rectangular walls and one core plate (Figure S1). TEM images reveal dimensions of 26.1 ± 2.5 nm in height, 34.5 ± 2.9 nm in outer width and 25.4 ± 1.8 nm in inner width (Figure S8). The experimental data agree with the calculated dimensions of the lidless box, namely 25.4 nm, 31.7 nm and 21.3 nm (Figure S1C), respectively.

4. Incorporation of Gold Nanospheres into Pandora's Box

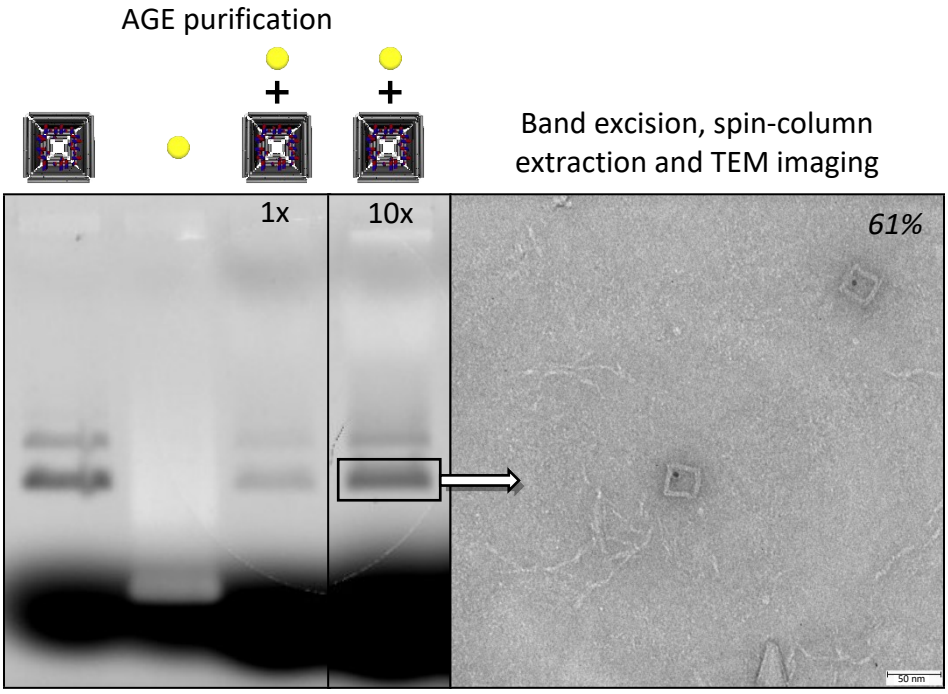

Figure S10. AGE purification of conjugates containing AuNP and Pandora's Box (left). Stained TEM images of isolated Box-AuNP conjugates after spin-column extraction (right). Italic numbers indicate the resulting incorporation yield. For statistical analysis at least n = 244 boxes were counted. Scale bar: 50 nm.[2]

After incorporation, excess AuNP were removed by AGE purification as described above and conjugates were characterised by TEM (Figure S10). Yields of up to 77% were obtained.

5. Zelos

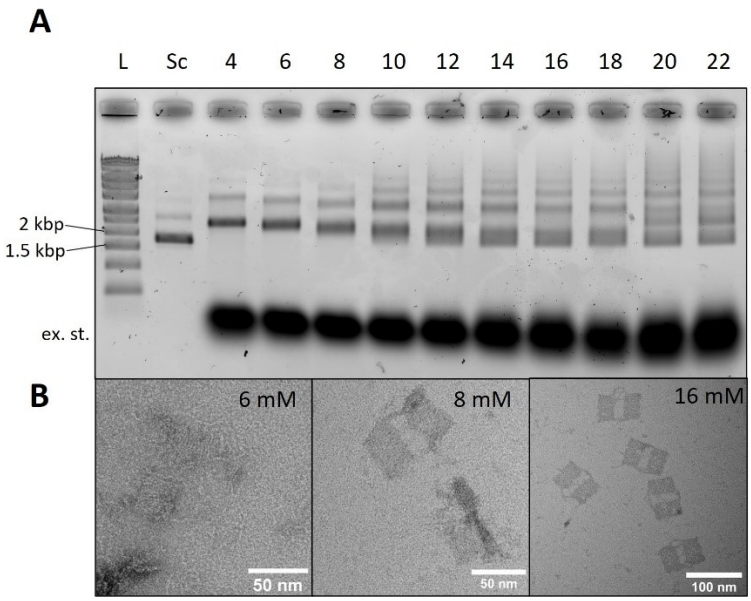

Figure S11. Magnesium-screening of Zelos assembly; L: 1 kbp ladder, Sc: scaffold, 4-22: $MgCl_2$ concentration used during assembly in mM, ex. st.: excess staple strands.[2]

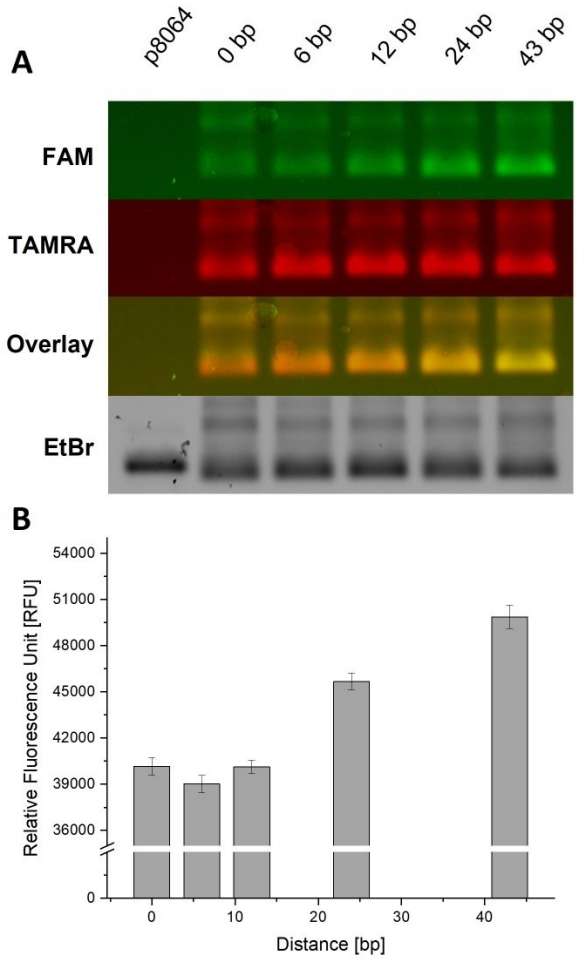

Figure S12. FRET-based determination of the adjustable distances of Zelos. (A) AGE analysis of Zelos labelled with FAM and TAMRA on each side, respectively. Lower gap distances lead to increased FRET extinction of the FAM fluorescence. Fluorophore channels were imaged separately followed by EtBr staining and imaging. (B) Relative FAM fluorescence intensity with different gap distances of Zelos.[2]

6. Hybridisation of Pandora's Box onto Zelos

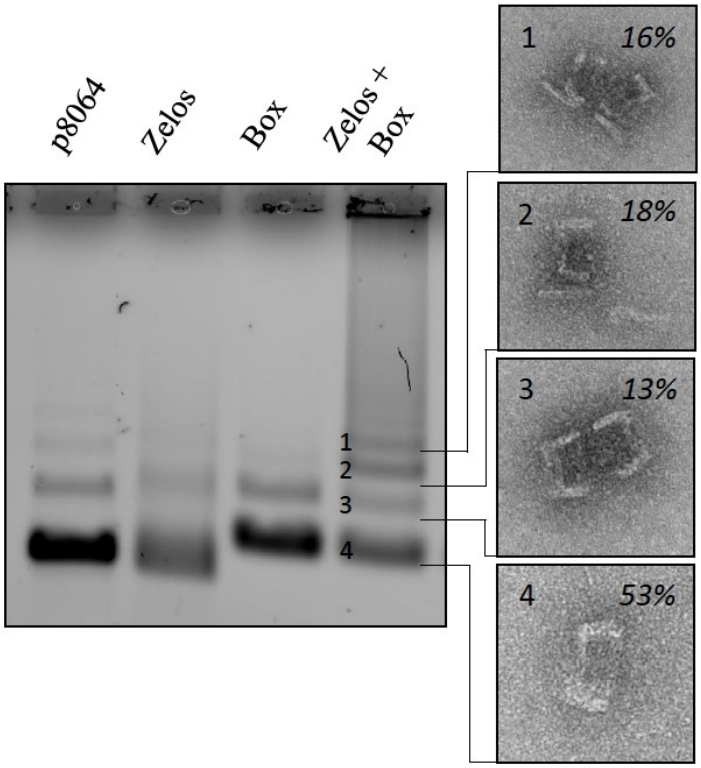

Figure S13. AGE (left) of individual DNA origamis as well as the hybridization sample of Zelos and Box. Hybridization was performed for seven days at 30 °C, 11 mM MgCl2 in a 4:1 (Box:Zelos) stoichiometry. Two new bands appear (1 and 2). TEM analysis (right) revealed the identity of each band. Percentages give information about the yield of each species, calculated from the EtBr intensity. The agarose gel was run at 80 V for 2 hours in an ice bath.

References

1. J. E. Park, Y. Lee and J. M. Nam, *Nano Lett*, 2018, **18**, 6475-6482.
2. M. Erkelenz, PhD thesis, *Dynamic DNA origami-based gold nanoparticle dimerization: design and assembly of a hybrid platform for nanophotonics*, University of Duisburg-Essen, 2021.
3. P. B. Johnson and R. W. Christy, *Physical Review B*, 1972, **6**, 4370-4379.