

# The Assemble, Grow and Lift-Off (AGLO) Strategy to Construct Complex Gold Nanostructures with Pre-Designed Morphologies

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## Supplementary Information

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## S-I. Materials

Agarose (electrophoresis grade, cat. # AGA002), tris(hydroxymethyl)-aminomethane (Tris), urea, boric acid and ethylenediaminetetraacetate (EDTA) were purchased from Bioshop Canada Inc. and used as supplied. Glacial acetic acid, sodium chloride, sodium hydroxide, ammonium hydroxide and hydrogen peroxide (30%) were purchased from ACP chemicals. MilliporeSigma™ Amicon™ Ultra Centrifugal Filter Units were used as received from Fisher Scientific. Dynabeads™ MyOne™ Carboxylic Acid (MB, cat. # 65011) was purchased from ThermoFisher Scientific. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl) was purchased from AK Scientific. Sephadex G-25 (super fine, DNA grade) was purchased from Glen Research. Gold (III) chloride trihydrate ( $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ , cat. # 520918), PEG acid disulfide ( $n=7$ , cas # 873013, OEG), HEPES (cat. # H3375), polyvinylpyrrolidone (PVP360 and PVP10), magnesium chloride hexahydrate and Bis(p-sulfonatophenyl)phenylphosphine dihydrate dipotassium salt (BSPP) were purchased from Sigma-Aldrich and used as received. Carbon-coated TEM grids were purchased from Electron Microscopy Sciences and ruby mica sheets (V1/V2 quality, grade 2) were purchased from S&J Trading and used for all AFM imaging. Milli-Q water was used for all aqueous solutions. 1×TBE buffer is composed of 90 mM Tris, 90 mM boric acid, 1.1 mM EDTA with a pH of  $\sim 8.3$  and 1×TBENa buffer contains additional 0.1 M NaCl. 1×TAMg buffer is composed of 40 mM Tris, 12.5 mM  $\text{MgCl}_2$ , with pH adjusted to  $7.8 \pm 0.1$  using glacial acetic acid. 10×HEPESMg buffer contains 1 M HEPES and 125 mM  $\text{MgCl}_2$  with pH adjusted to  $7.4 \pm 0.1$  using sodium hydroxide. 1×HEPESMg buffer was diluted 10 times from the concentrated buffer and used for all gold growth experiments. 1×HEPESNa buffer contains 0.1 M HEPES and 0.1 M NaCl with pH adjusted to  $7.4 \pm 0.1$  using sodium hydroxide.

For in-house DNA synthesis, the following materials and reagents were used. Pre-packed Controlled Pore Glass (CPG) solid support ( $1000\text{\AA}$  1  $\mu\text{mole}$  universal CPG, cat. # MM1-3500-1) columns and standard deoxyribonucleoside phosphoramidites for automated DNA synthesis were used as purchased from BioAutomation. Acetonitrile anhydrous, activator (0.25 M ETT in acetonitrile), oxidizer (0.02 M  $\text{I}_2$  in THF/pyridine/ $\text{H}_2\text{O}$ ), Cap A mix (THF/lutidine/ $\text{Ac}_2\text{O}$ ), Cap B mix (16% MeIm in THF), and deblock solution (3% DCA/DCM). DMT-Hexaethylene-Glycol phosphoramidite (HEG, cat. # CLP-9765) and Fmoc-Amino-DMT C3 CED phosphoramidite (cat. # CLP-1661) were purchased from ChemGenes Corporation. Origami M13mp18 FOUNDATION™ ssDNA Scaffold (7249) was purchased from Guild BioSciences. Origami staple strands were purchased from Bioneer and Integrated DNA Technologies.

## **S-II. Instrumentation and sample preparation**

### **1. Instrumentation**

Standard DNA oligonucleotides synthesis was carried out on a BioAutomation MerMade MM6 DNA synthesizer. DNA quantification measurements were performed by UV absorbance on a NanoDrop™ Lite spectrophotometer from Thermo Scientific. Bio-Rad T100™ thermal cycler and ProFlex™ 3×32-well thermal cycler were employed for all annealing and heated incubation processes. Agarose gel electrophoresis (AGE) experiments were performed on a Thermo Scientific™ Owl™ EasyCast™ B1 Mini Gel Electrophoresis System. Gel images were captured using a ChemiDoc™ MP System from Bio-Rad Laboratories. BioTek Synergy HT Multi-Detection Microplate Reader was used for the quantification of gold nanoparticle (AuNP) and its assemblies. Centrifugation for AuNP workup was performed on an Eppendorf 5702R Centrifuge while centrifugation for sample washing in the Amicon Ultra 0.5 mL centrifugal filters (100 kDa) was performed on a Sorvall™ Legend™ Micro 21 Centrifuge. DLS experiments were carried out using a DynaPro™ Instrument from Wyatt Technology. Atomic absorption spectroscopy (AAS) measurements were conducted on a Perkin Elmer AAnalyst 800 atomic absorption spectrometer and NMR experiments on a Bruker AVIIIHD 500 MHz NMR Spectrometer. Multimode 8 scanning probe microscope and Nanoscope V controller (Bruker) were employed to acquire AFM images. TEM images were acquired using the Philips CM200 (200 kV) TEM instrument and the FEI Tecnai G2 Spirit Twin 120 kV TEM.

### **2. AFM and TEM sample preparation**

Samples for AFM imaging were prepared by depositing 5 µL of the sample (5 nM origami) onto a freshly cleaved mica surface for 30 seconds, followed by three times washing with 50 µL of Millipore water. Excess liquid was blown off and dried by a stream of compressed air for 30 seconds. The sample was then put under vacuum for at least 2 hrs prior to imaging. AFM images were acquired in ScanAsyst mode under dry conditions using ScanAsyst-Air silicon tip on nitride lever (tip radius = 2 nm,  $k = 0.4$  N/m,  $f_0 = 70$  kHz; Bruker).

Samples for TEM imaging were generally prepared by depositing 5 µL of the sample solution onto the carbon-coated Cu grid. After 1 min, the excess sample solution was removed using a filter paper. For the samples of the washed crude AGLO product dispersed in water, three small droplets of the solution were dropped onto the TEM grid separately and left in air (undisturbed) to dry. Finally, all sample grids were put under vacuum for 16 hours prior to imaging.

### S-III. DNA synthesis and DNA sequences

#### 1. DNA synthesis

DNA synthesis was performed through standard automation procedures on a 1  $\mu$ mole scale, using a universal 1000 Å CPG solid support. Briefly, the phosphoramidite was activated by 0.25 M 5-(ethyl)-1*H*-thiotetrazole in acetonitrile and then coupled to DNA chains on the solid support. Failed coupling was subsequently capped by THF/lutidine/acetic anhydride and 16% 1-methylimidazole/THF. Phosphorus (III) was oxidized to phosphorus (V) with 0.02 M  $I_2$  in THF/pyridine/ $H_2O$ . Coupling efficiency was monitored after the removal of the dimethoxytrityl (DMT) 5'-OH protecting groups with 3% dichloroacetic acid in dichloromethane. Non-nucleoside phosphoramidites used on the DNA synthesizer, such as the DMT-hexaethyloxy-glycol phosphoramidite (HEG), were firstly dissolved in acetonitrile to obtain a 0.1 M solution under the nitrogen atmosphere in the glove box, and then loaded to the DNA synthesizer. The coupling time was extended to 5 minutes for better coupling yield. For the off-column coupling of the DMT dithiol phosphoramidite (DTPA)<sup>1</sup> and the Fmoc-Amino-DMT C3 CED phosphoramidite, the phosphoramidites were initially diluted with acetonitrile to a concentration of 0.1 M in a glove box. A 10-fold excess of the phosphoramidites was manually coupled to the DNA sequence with an equimolar amount of ethylthiotetrazole (0.1 M in acetonitrile) for an extended reaction time of 10 minutes with the help of a syringe adaptor for the CPG column in the glove box. After coupling, the CPG column was removed from the glove box and re-loaded to the DNA synthesizer for oxidation, capping and deblock procedures as in conventional automated solid-phase DNA synthesis.

After the synthesis cycles, the completed DNA strands were deprotected in 28% ammonium hydroxide solution for 16-18 hrs at 65 °C. The solid support was then removed from the crude deprotected DNA solution and the solution was dried under reduced pressure at 60 °C and then resuspended in 1:1  $H_2O$ /8 M urea before loaded to a 12% PAGE/urea gel (one-third of the crude 1  $\mu$ mol synthesis product was loaded per gel) for purification. The gel was run at a constant current of 30 mA for 1.5 hrs (30 min pre-run at 250V, followed by 1 hr at 500 V) with 1xTBE buffer. The product bands were excised under UV light on silica plates and the DNA product was extracted by crushing and soaking the cut gel pieces in 10 mL Millipore water at 65 °C for 16-18 hrs. The solution was separated and concentrated to approximately 1 mL and then desalted using size exclusion chromatography (Sephadex G-25 column), and quantified using UV-Vis spectroscopy at 260 nm. DTPA functionalized DNA strands were used directly after synthesis to functionalize gold nanoparticles (AuNPs) without gel purification, as the DTPA unit is at the 5' end and only the full length sequence can conjugate to the AuNP surface through the disulfide of the DTPA.



## 2. DNA sequences

Non-staple DNA strand sequences used in this paper are listed in **Table S1**. BS1 and BS2 strands are the 5'-DTPA functionalized binding strands used to conjugate to the AuNP seeds. BS1 comp and BS2 comp are the full complementary strands acting as invading strands during strand displacement procedures. LS strand is the 3'-amino group modified linking strand used to conjugate to the magnetic beads (MBs) to anchor the AuNP seed-DNA origami assemblies. DNA origami staple strands and their modifications are listed and discussed in section S-V.

**Table S1. Non-staple DNA strand sequences used in this paper.**

Strand	Sequence (5' → 3')
BS1	<b>DTPA</b> -GTCGCTCTCTCAAGTAGAAT
BS2	<b>DTPA</b> -TTTATATGGTCAACTGAAA
BS1 comp	ATTCTACTTGAGAGAGCGAC
BS2 comp	TTTCAGTTGACCATATAAA
LS	<b>NH<sub>2</sub></b> - <b>HHHH</b> TTTTTTTTTTGTGGCGGCGGTGCCGTAATTGCTACCG

\***DTPA** is the cyclic disulfide modification<sup>1</sup>, **NH<sub>2</sub>** is the amino group modification and **H** is the hexaethylene glycol spacer modification.

## **S-IV. Gold nanoparticle preparation**

### **1. Gold nanoparticle synthesis**

The 12 nm AuNP seeds used in this work were prepared via a modified approach described in a previous work<sup>2</sup>. Briefly, 10% H<sub>2</sub>AuCl<sub>4</sub>·3H<sub>2</sub>O (w/v) Milli-Q water solution (1 mL) was added to 300 mL Milli-Q water in a clean flask. The solution was brought to a boil with vigorous stirring under reflux. 12 mL trisodium citrate (0.1 M) was then quickly injected to the boiling solution and the solution color will change to dark red in seconds. The reaction mixture was allowed to continue to boil for 10 min after the color change and then cooled down to room temperature.

After cooling the reaction solution to room temperature, 0.2 g BSPP was added to the solution and stirred overnight to improve AuNP stability. Subsequently, the AuNPs were precipitated out of solution via the addition of NaCl and centrifugation at 4400 rpm for 10 min. The supernatant was removed, and the AuNPs resuspended in 0.25 mM BSPP/water solution. The monodispersity of the AuNPs was further increased by running on a 3% agarose gel at 120 V for 1-3 hrs and excising the front half fractions of the gel band, followed by gel electroelution to recover the monodispersed AuNPs. The purified AuNPs were quantified on a plate reader using the extinction coefficient ( $\epsilon_{450} = 9.59 \times 10^7$ ) reported in the literature.<sup>3</sup>

### **2. Gold nanoparticle conjugation**

AuNPs functionalized with BS1 and BS2 strands were prepared by heating purified 12 nm AuNPs (20 to 50 pmol) with 100 equiv. BS1 or BS2 strands in 1×HEPESNa buffer (total volume 50 to 100  $\mu$ L) to 50 °C for 1 hr and cooling down to 20 °C over 30 min in a thermal cycler. Then, 40,000 equiv. OEG ligand was added to the solution and incubated for 30 min at room temperature to further passivate the unprotected surface of the AuNP. Next, the poly-conjugated AuNP seeds were washed 5 times with fresh 1×HEPESNa buffer in an 100 kDa Amicon Ultra 0.5 mL centrifugal filter Unit to remove the DNA strands and OEG in excess and then re-quantified on the plate reader based on the absorbance at 450 nm.

## **S-V. DNA origami design, staple strand modifications and AuNP seed-origami assembly**

### **1. DNA origami template assembly**

The assembly of the DNA origami template was based on the method reported by Rothemund<sup>4</sup>. The long circular single-stranded viral scaffold M13mp18 (purchased from Guild BioSciences, USA) was folded into a rectangular tile with the aids of multiple short single-stranded staple strands (purchased from Bioneer, Inc., USA). Staple strand sequences are listed below.

DNA origami templates were assembled in one-pot, where 3 nM M13mp18 scaffold and 45 nM each staple strands were heated to and held at 95 °C for 5 min in 1×HEPESMg buffer and slowly annealed to 20 °C (-1 °C/min). To remove excess staple strands, the annealed sample was purified with a 100 kDa Amicon centrifugal filter Unit. First, 500  $\mu$ L samples were centrifuged at 6500 rpm at 4 °C for 5 min. Then, 400  $\mu$ L 1×HEPESMg was added and the samples were centrifuged again at 5000 rpm at 4 °C for 6 min. This filtration step was repeated three more times. Approximately 50-100  $\mu$ L samples were recovered after the purification.

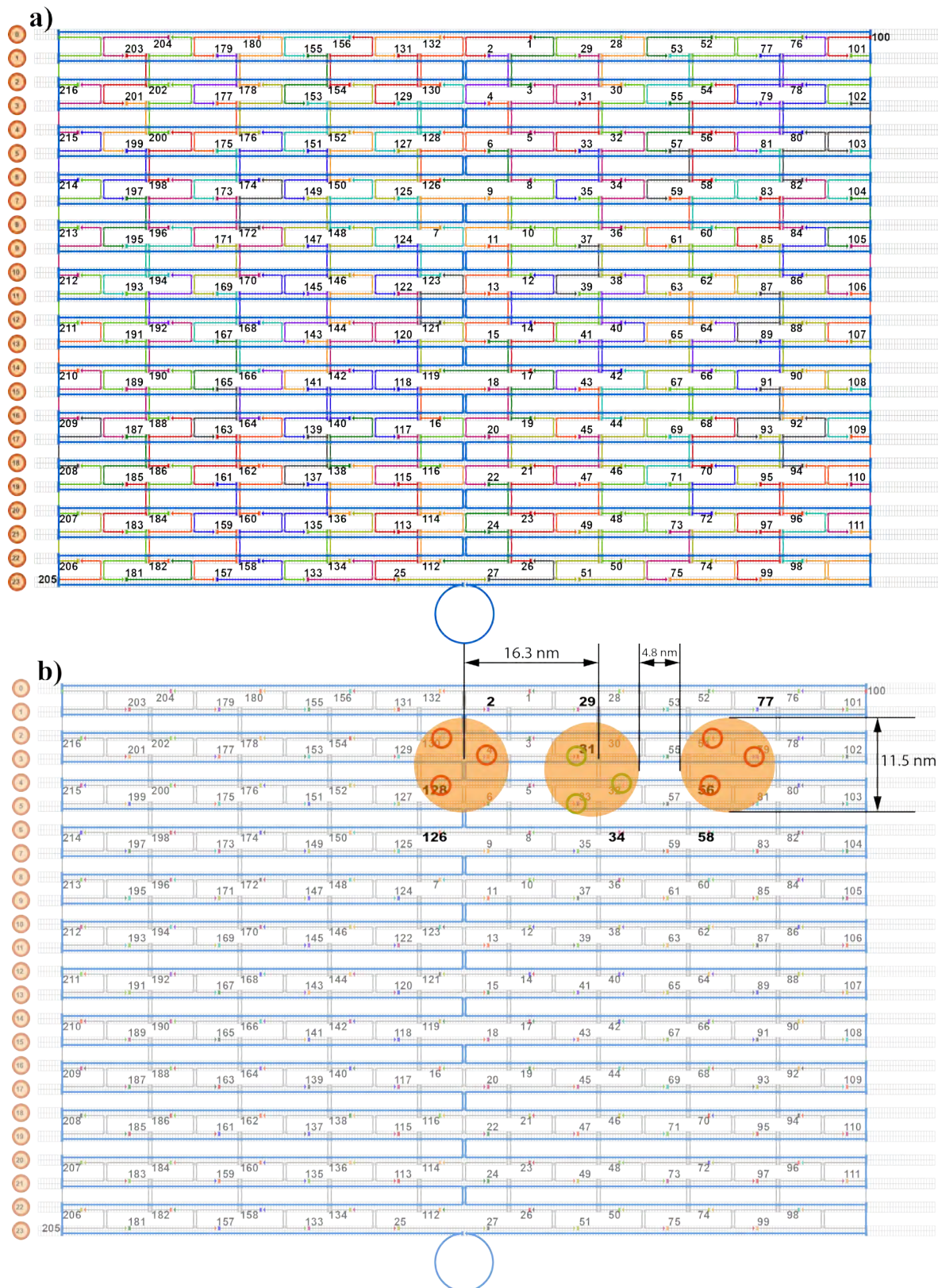
To determine the concentrations of the DNA origami templates, their absorbance at 260 nm was measured by NanoDrop Lite Spectrophotometer (ThermoFisher Scientific). The extinction coefficient of each DNA origami template design can be approximated by equation (1)<sup>5</sup>

$$\varepsilon = 6700ds + 10000ss \quad (1)$$

where ds is the number of double stranded base pairs and ss is the number of single-stranded bases. Using Lambert-Beer's law ( $A_{260 \text{ nm}} = \varepsilon bc$ ,  $b = 1 \text{ cm}$ ), the concentrations of DNA origami templates can be calculated.

### **2. DNA origami design and staple strand modification**

The detailed single-layered rectangular DNA origami design is demonstrated in **Fig. S1a** where original staple strand 1-216 are marked at their 5' end. Original staple strand sequences are listed at the end of this file.



**Fig. S1. a)** The basic design of the single-layered rectangular DNA origami template. Original staples strand sequences are listed at the end of this supporting information. Modified staples strand sequences are listed in **Table S2. b)** The design details of the assembly of the AuNP Linear Trimer structure. Binding sequences are extended from the 3' ends (Red/green circles) of the original staple strands (number highlighted). AuNPs are at the back of the origami plane. Binding site distances estimated by DNA base pairs (0.34 nm / base pair).

In order to anchor BS1 or BS2 strand functionalized AuNP seeds, some original staple strands are specifically chosen to be replaced by their extended version with BS1' or BS2' sequence at the 3' ends for the origami assembly. For example, to construct the Linear Trimer structure, staple strand 2, 126, 128, 29, 31, 34, 56, 58 and 77 (**Fig. S1b**, highlighted numbers) are replaced by 2BS1', 126BS1', 128BS1', 29BS2', 31BS2', 34BS2', 56BS1', 58BS1' and 77BS1', which are further annealed with the M13mp18 scaffold and the other original staple strands to form the DNA origami template. Similarly, for AGLO-MB experiments, staple strand 101, 110 and 215 are replaced by LS'101, LS'110 and LS'215 to anchor the DNA origami template onto the MB surface. A full list of modified staple strand sequences is shown below in **Table S2**.

**Table S2. Modified staple strand sequences used in this paper.**

Strand	Sequence (5' → 3')
2 BS1'	AATGCCCCGTAACAGTGCCCGTATCTCCCTCATTCTTGAGAGAGCGAC
126 BS1'	GAATAAGGACGTAACAAAGCTGCTCTAAAACATTCTTGAGAGAGCGAC
128 BS1'	CTCATCTTGAGGCAAAAGAATACAGTGAATTTTTCTTGAGAGAGCGAC
56 BS1'	TCGGCATTCCGCCGCCAGCATTGACGTTCCAGTTTCTTGAGAGAGCGAC
58 BS1'	TCACAATCGTAGCACCATTACCATCGTTTTTCATTCTTGAGAGAGCGAC
77 BS1'	TGCTCAGTCAGTCTCTGAATTTACCAGGAGGTTTTCTTGAGAGAGCGAC
68 BS1'	AAATCAATGGCTTAGGTTGGGTACTAAATTTTTCTTGAGAGAGCGAC
70 BS1'	AACCTACCGCGAATTATTCATTTCCAGTACATTTCTTGAGAGAGCGAC
89 BS1'	AGAGGCATAATTTTCATCTTCTGACTATAACTATTTCTTGAGAGAGCGAC
15 BS1'	GCGTTATAGAAAAAGCCTGTTTAGAAGGCCGTTTCTTGAGAGAGCGAC
16 BS1'	GCTCATTTTCGCATTAAATTTTTGAGCTTAGATTTCTTGAGAGAGCGAC
116 BS1'	TTCGCCATTGCCGGAACACAGGCATTAAATCATTCTTGAGAGAGCGAC
29 BS2'	CTGAAACAGGTAATAAGTTTTAACCCTCAGATTTGTTGACCATATAAA
31 BS2'	GCCACCACTCTTTTCATAATCAAACCGTCACCTTTGTTGACCATATAAA
34 BS2'	AGCGCCAACCATTTGGGAATTAGATTATTAGCTTTGTTGACCATATAAA
64 BS2'	AATGCAGACCGTTTTTATTTTCATCTTGCGGGTTTGTGACCATATAAA
83 BS2'	TTATTACGGTCAGAGGGTAATTGAATAGCAGCTTTGTTGACCATATAAA
85 BS2'	CTTTACAGTTAGCGAACCTCCCGACGTAGGAATTTGTTGACCATATAAA
41 BS2'	ACGCTCAAATAAGAATAAACACCGTGAATTTTTGTTGACCATATAAA
43 BS2'	ATCAAAATCGTCGCTATTAATTAACGGATTCGTTTGTGACCATATAAA
46 BS2'	ACAGAAATCTTTGAATACCAAGTTCCTTGCTTTTTGTTGACCATATAAA
9 BS2'	AAAAGTAATATCTTACCGAAGCCCTTCAGAGTTTGTGACCATATAAA

121 BS2'	TTTCATTTGGTCAATAACCTGTTTATATCGCGTTTGTGACCATATAAA
123 BS2'	TTTAAATTGCCCGAAAGACTTCAAAACACTATTTTGTGACCATATAAA
18 BS2'	TTAAGACGTTGAAAACATAGCGATAACAGTACTTTGTGACCATATAAA
114 BS2'	GCATAAAGTTCCACACAACATACGAAGCGCCATTTGTGACCATATAAA
116 BS2'	TTCGCCATTGCCGGAACCAGGCATTAAATCATTGTGACCATATAAA
122 BS1'	TCGCAATGGGGCGCGAGCTGAAATAATGTGTTTTCTTGAGAGAGCGAC
140 BS1'	AAATAATTTTAAATTGTAAACGTTGATATTCATTCTTGAGAGAGCGAC
142 BS1'	ACCGTTCTAAATGCAATGCCTGAGAGGTGGCATTCTTGAGAGAGCGAC
17 BS1'	AATTACTACAAATTCTTACCAGTAATCCCATCTTTCTTGAGAGAGCGAC
19 BS1'	TAGAATCCCTGAGAAGAGTCAATAGGAATCATTTTCTTGAGAGAGCGAC
39 BS1'	GGTATTAAGAACAAGAAAAATAATTAAAGCCATTTCTTGAGAGAGCGAC
LS'101	CGGTAGCAATTACGGCACCGCCGCTTTTTTAGGGTTGATTTTATAAATCCTCATTAATGATATTC
LS'110	CGGTAGCAATTACGGCACCGCCGCTTTTTTGATGGCAATTTTAATCAATATCTGGTCAAAATATC
LS'215	CGGTAGCAATTACGGCACCGCCGCTTTTTTACGGTCAATTTTGACAGCATCGGAACGAACCCTCAG

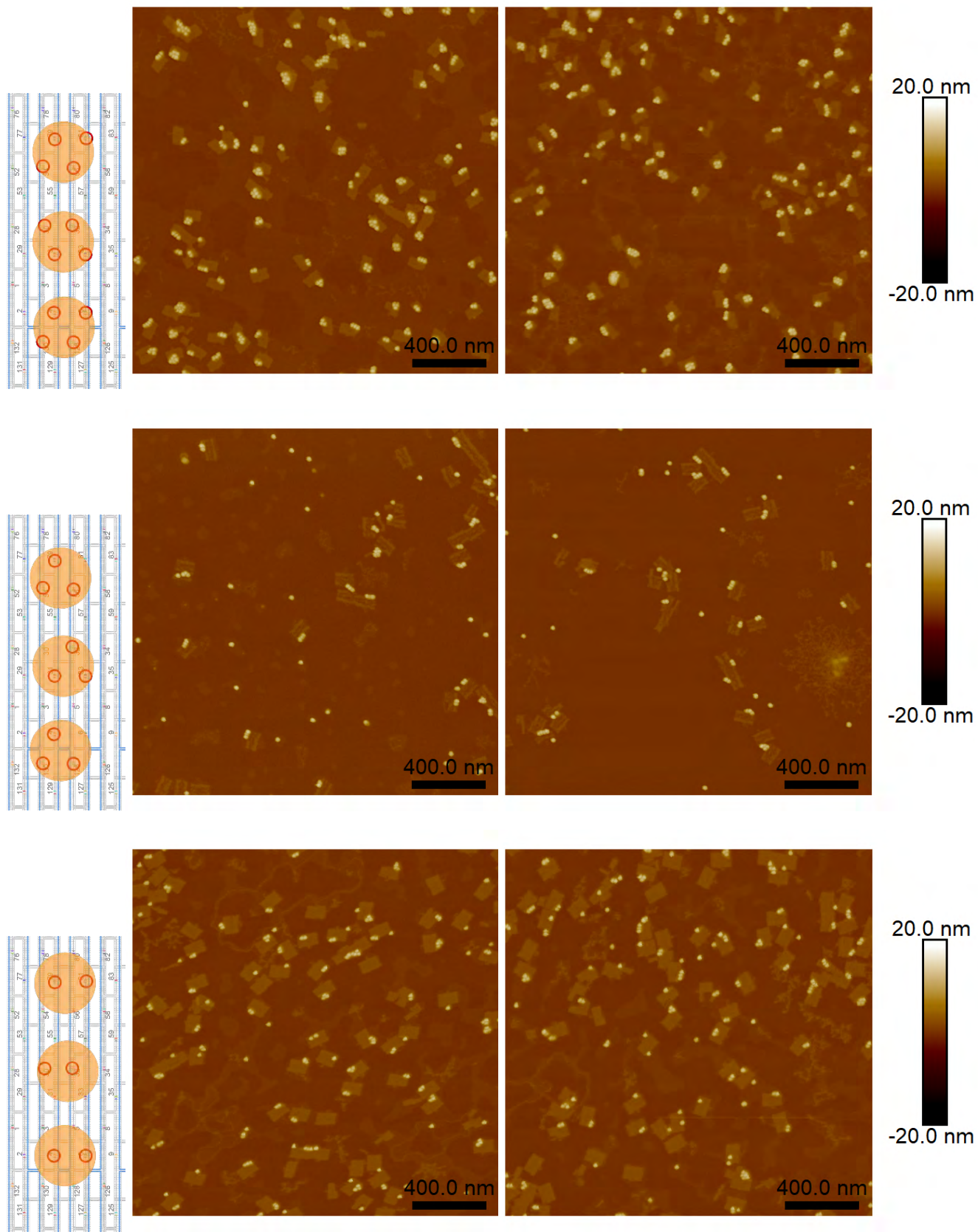
### 3. Assemble AuNP seeds to DNA origami template

To assemble AuNP seeds to the DNA origami template, 5 nM DNA origami was slowly added to concentrated AuNP-BS2 and mixed well with a pipette; after 5 min incubation at room temperature, concentrated AuNP-BS1 was added subsequently to the mixture and mixed with a pipette. The mixture was then slowly annealed over night from 37 °C to 10 °C (incubate at 37 °C for 30 min, cool down to 20 °C with a rate of 11 min/°C; then heat to 30 °C and incubate for 30 min, followed by cooling down to 10 °C with the same rate). For experiments without agarose gel purification of the excess AuNPs before the gold growth, 1.5 equiv. of AuNP-BS2 and 1.2 equiv. of AuNP-BS1 (equivalent to the number of binding sites) were used. For experiments with a following agarose gel purification step (**Fig. S6**) before the gold growth step, 7 equiv. of both AuNP-BS2 and AuNP-BS1 were applied to further increase the assembly yield.

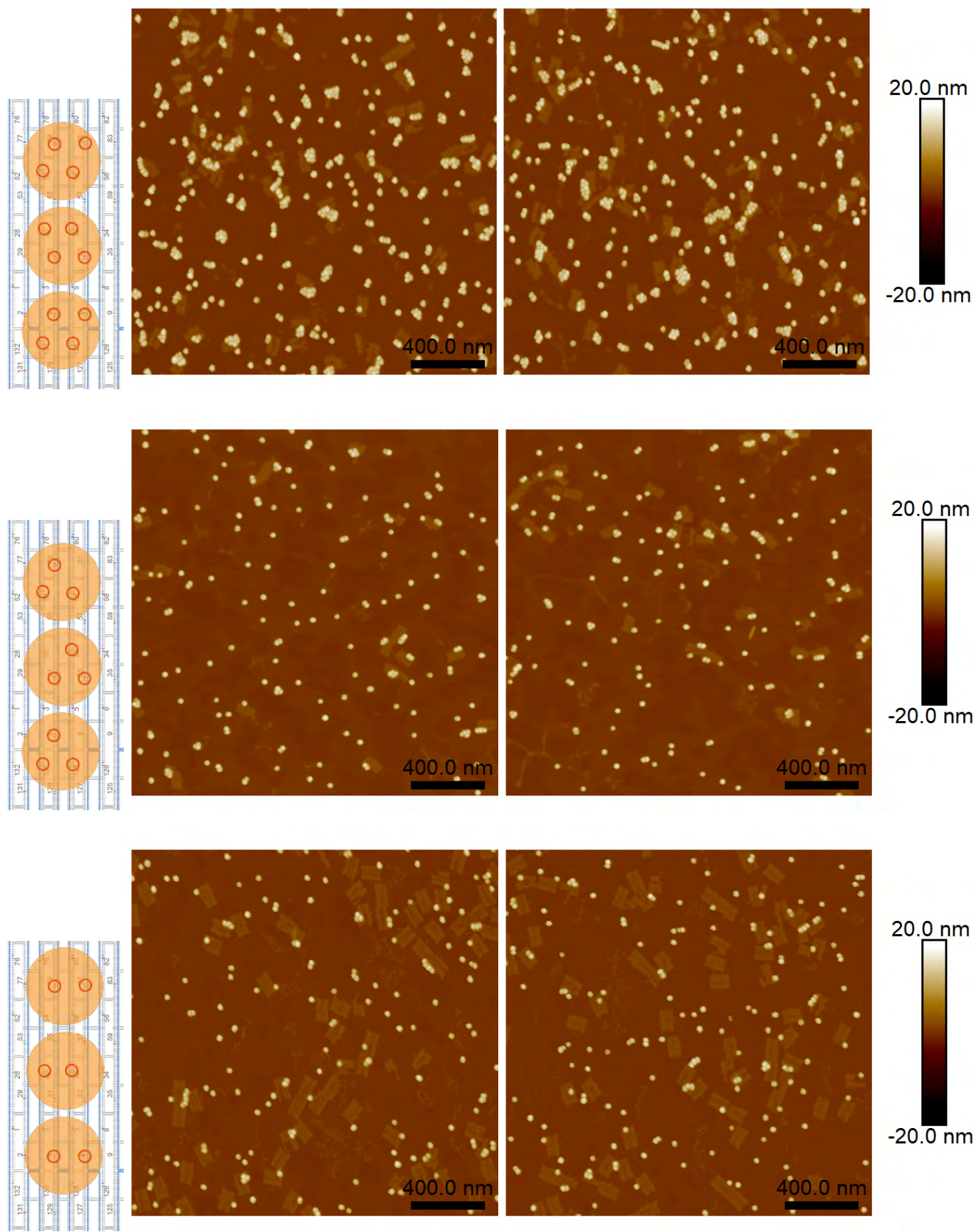
To optimize the assembly yield of the Linear Trimer seed-origami structure, a different number (2 to 4) of binding strands per binding site, different sizes (12 nm or 15 nm) of the AuNPs and different binding site designs (inter-binding site distance, whether to use different binding sequences) were applied to find the parameters with the highest assembly yield (**Fig. S2**). The results show that assembling ~11.5 nm AuNP seeds to the DNA origami template with binding sites that are 16.3 nm apart, containing 3 binding strands per binding site and having different binding sequences for neighboring sites, gives the highest assembly yield of the Linear Trimer structure.



a) The influence of the number of binding strands per binding site.

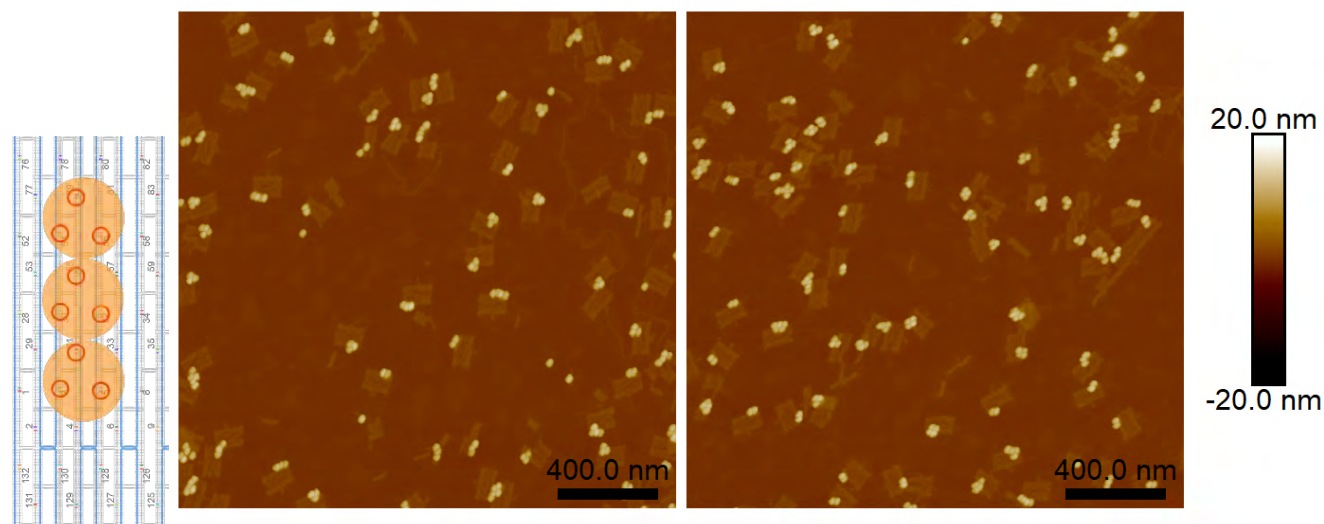


**b)** The influence of the AuNP size. 15 nm AuNPs were used instead of the normal 11.5 nm AuNPs.

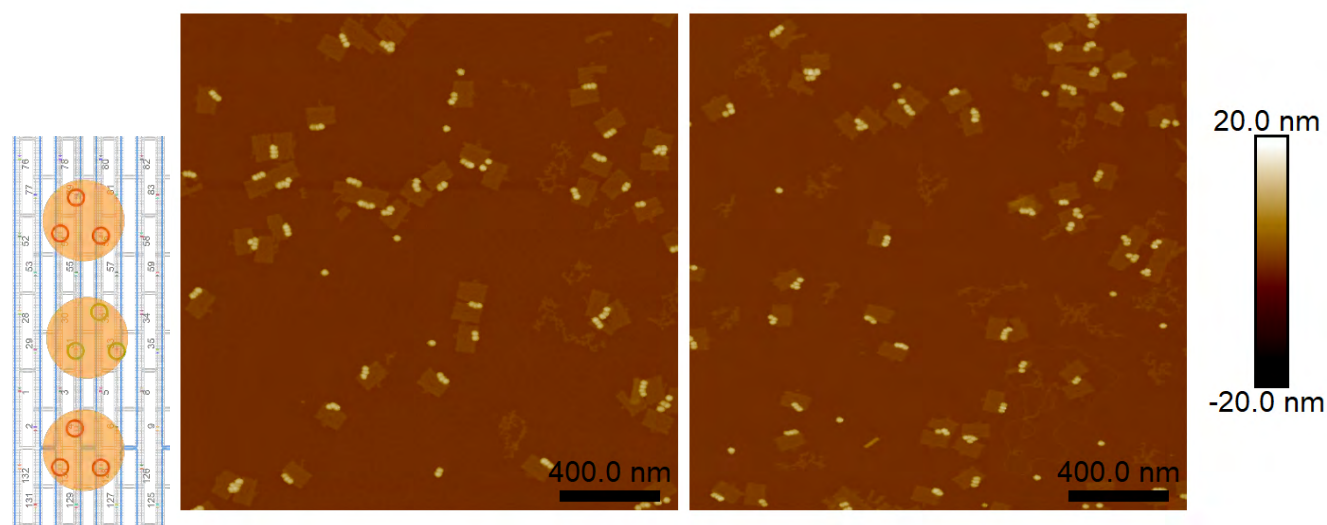




c) The influence of binding site distance. 11.9 nm binding site distance was used instead of 16.3 nm.



d) The influence of using different binding sequences for adjacent binding sites. (Red: BS1', Green: BS2')

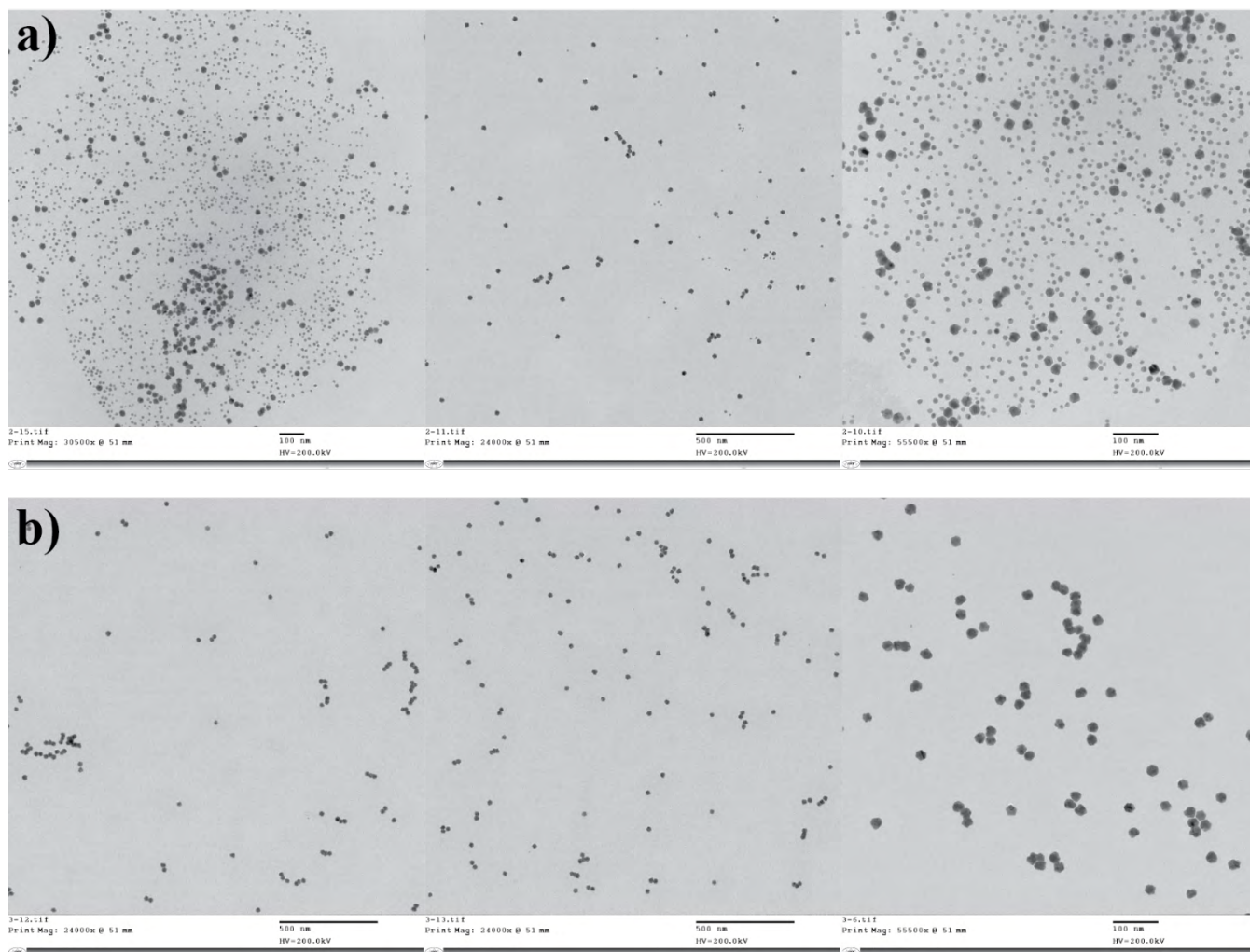


**Fig. S2.** Optimization of the AuNP Linear Trimer assembly on the DNA origami template. **a)** The influence of the number of binding strands per binding site. **b)** The influence of the AuNP size. **c)** The influence of binding site distance. **d)** The influence of using different binding sequences for adjacent binding sites. The experiments show that assembling  $\sim 11.5$  nm AuNP seeds to the DNA origami template with binding sites that are 16.3 nm apart, containing 3 binding strands per binding site and having different binding sequences for neighboring sites, gives the highest assembly yield of the Linear Trimer structure. 1.5 equiv. of AuNP-BS2 and 1.2 equiv. of AuNP-BS1 were used for all assemblies.

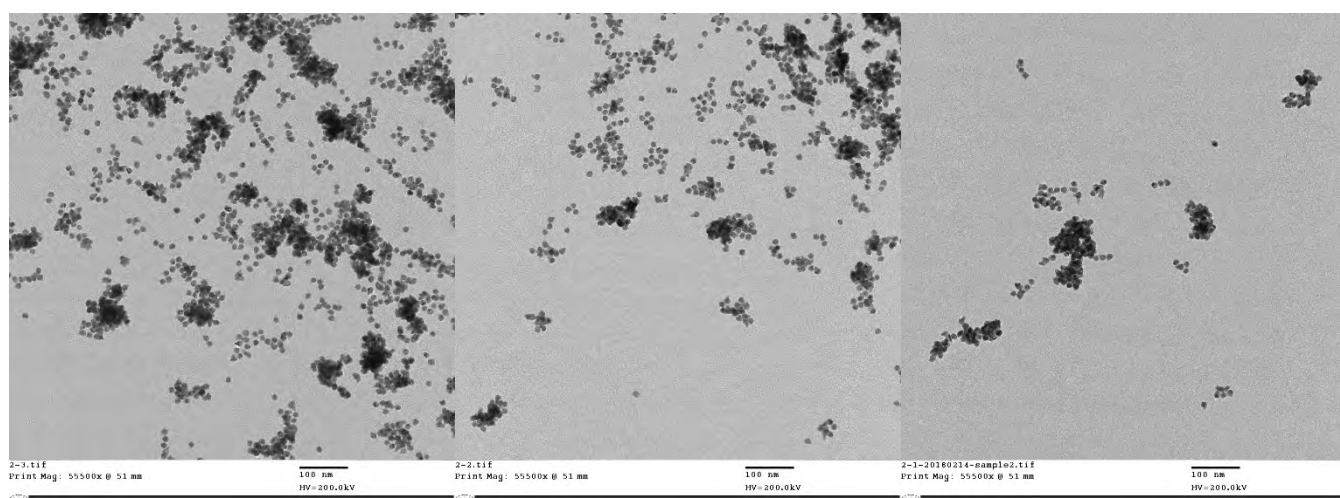
## **S-VI. Gold growth and Lift-Off.**

### **1. General procedure for unpurified Linear Trimer assembly.**

For the unpurified Linear Trimer assembly with 1.5 equiv. of AuNP-BS2 and 1.2 equiv. of AuNP-BS1, directly after the AuNP seed-origami assembly step, 20  $\mu\text{L}$  sample (containing 5 nM origami) was diluted with a solution containing 125  $\mu\text{L}$  1 $\times$ HEPESMg buffer, 10  $\mu\text{L}$  PVP360/ $\text{H}_2\text{O}$  (5g/100mL) and 5  $\mu\text{L}$  10 $\times$ HEPESMg buffer in an Eppendorf tube. The diluted seed-origami solution was then chilled in an ice bath for 5 min, followed by adding 40  $\mu\text{L}$  gold source (0.06 g/100 mL  $\text{HAuCl}_4/\text{H}_2\text{O}$  solution) and carefully mixing with a pipette. The solution was then left in the ice bath for 3 hrs without disturbance, during which time the solution color will change from light pink/red to dark purple/blue. Following the incubation, the Eppendorf was moved from the ice bath to room temperature and 5  $\mu\text{L}$  0.1 M OEG/ $\text{H}_2\text{O}$  solution was added and mixed by vortex. After a 30 min incubation, 200  $\mu\text{L}$  8 M urea solution was added to the Eppendorf to denature the DNA origami template and lift-off the gold nanostructure product. The crude product was then centrifuged and washed one more time with 8 M urea and 3 times with Milli-Q water. Ultrasonication treatment was conducted after each centrifugation to re-disperse the crude AGLO product in urea solution or Milli-Q water. As a control, 0.39 pmol AuNP-BS1 was applied to replace the seed-origami assembly in the above experiment and all other procedures and conditions were kept the same. The washed crude products were loaded on a 1% agarose gel in 1 $\times$ TBE buffer and run for 25 min under 80 V. Gel bands were excised and soaked out in water for TEM characterization.

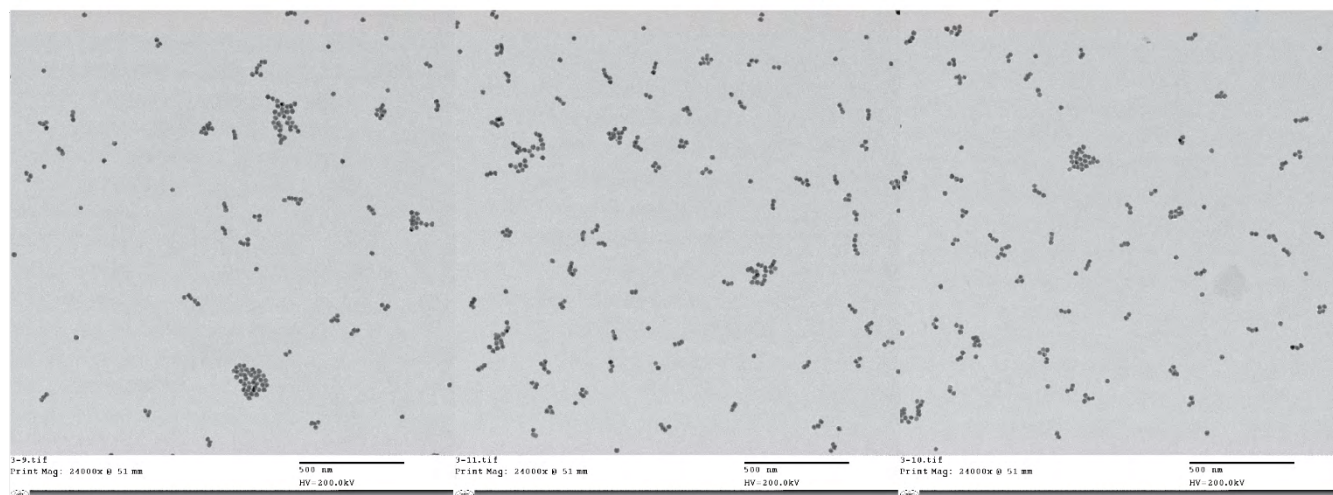
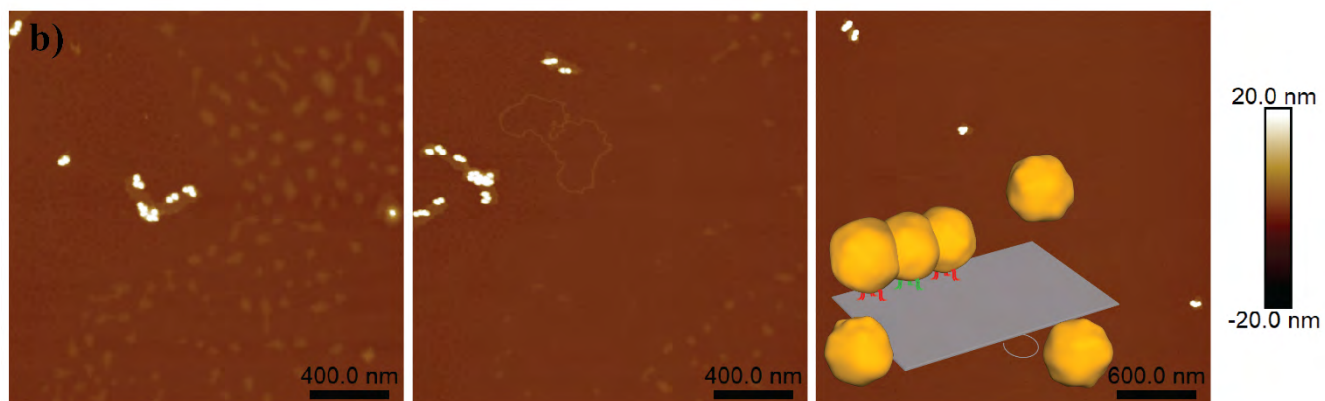
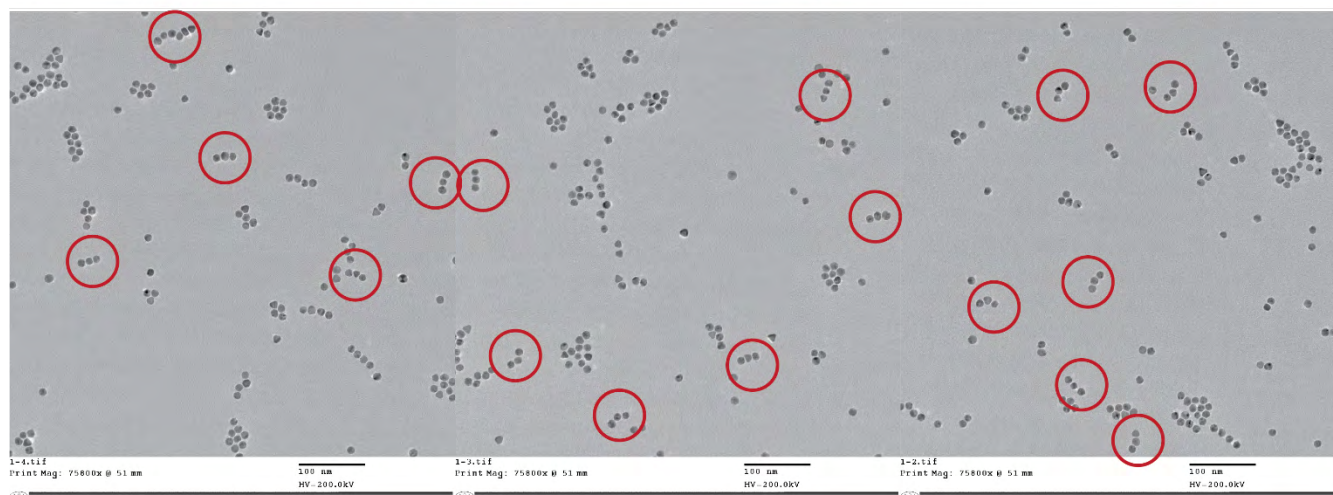
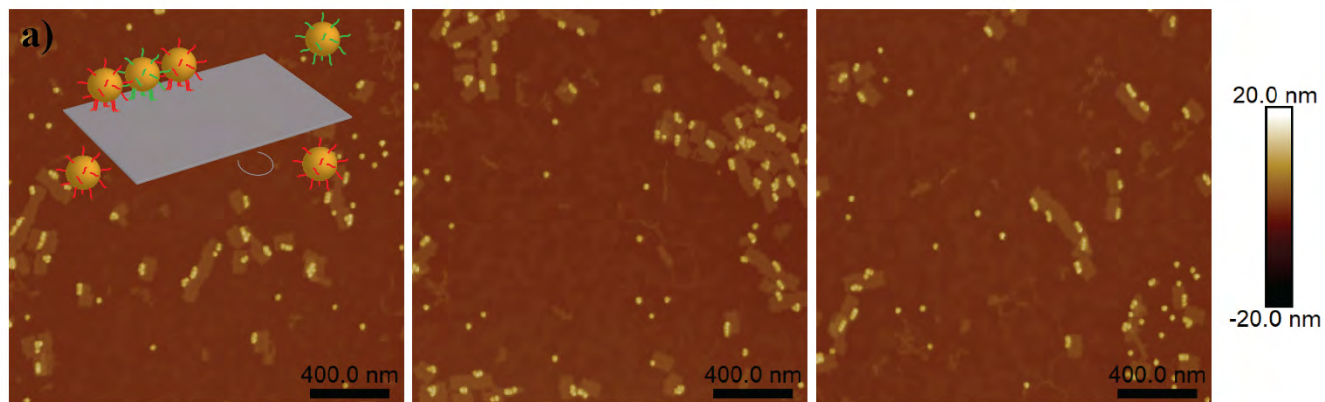


**Fig. S3.** The influence of the gold growth temperature. **a)** at room temperature; **b)** in an ice bath. 40  $\mu\text{L}$  0.06% gold source were used on 20  $\mu\text{L}$  5 nM AuNP-origami assembly (unpurified) in both experiments. Small AuNPs with a diameter less than 10 nm in **a)** are from random nucleation of the gold source in the bulk solution background.

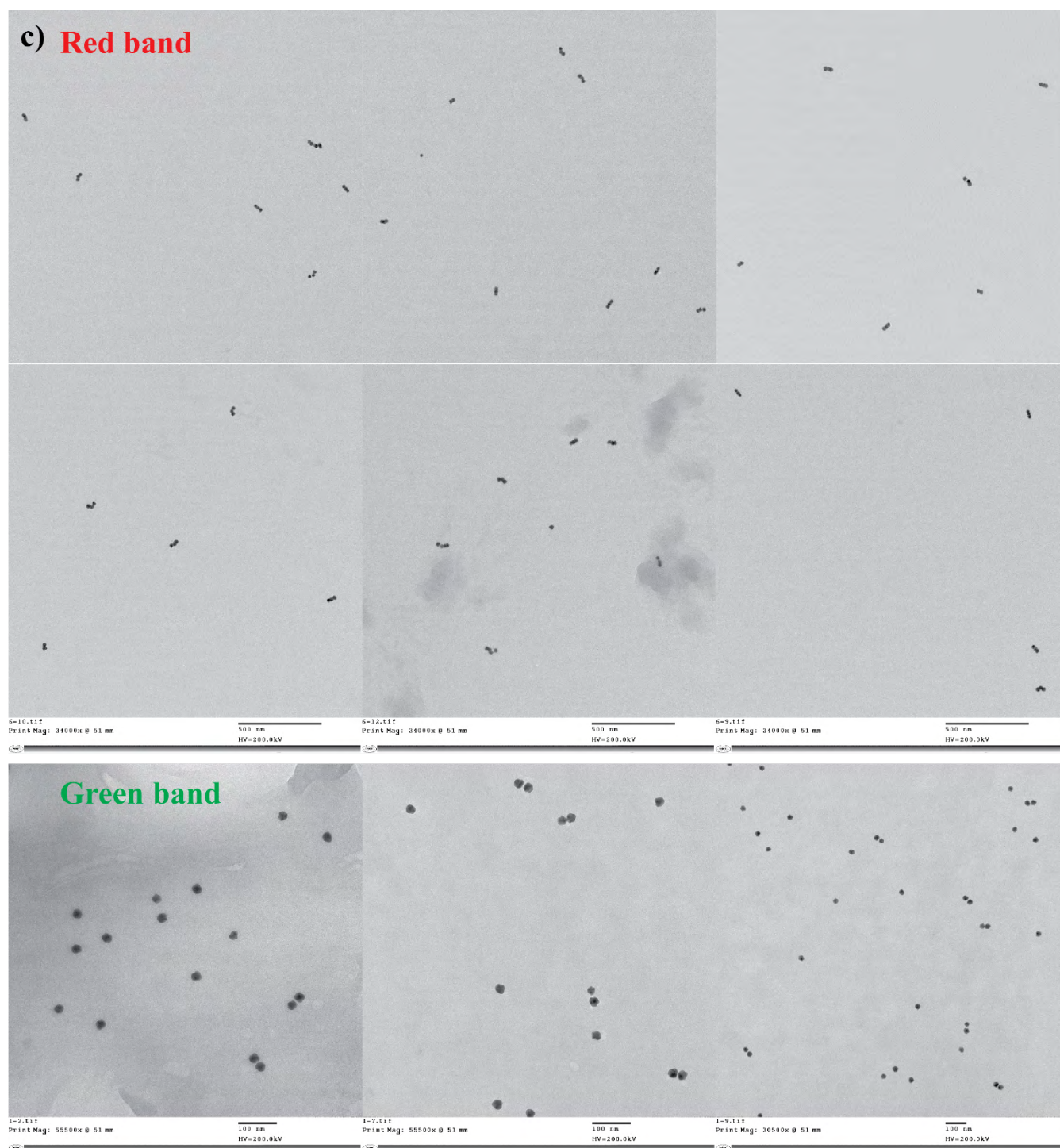


**Fig. S4.** Random aggregations during the gold growth step when no surfactant was applied, even with a small amount of gold source. 16  $\mu\text{L}$  0.06% gold source were used on 100  $\mu\text{L}$  2 nM AuNP-origami assembly (unpurified).









**Fig. S5.** Additional AFM and TEM images for **Fig. 2.** **a)** Images of the AuNP seed Linear Trimer assembly before gold growth. **b)** Images of the merged AuNP seed Linear Trimer assembly after gold growth. TEM images were acquired after OEG passivation and sample concentration. **c)** TEM images of the gel band extractions.

## 2. General procedure for all purified seed-origami assemblies

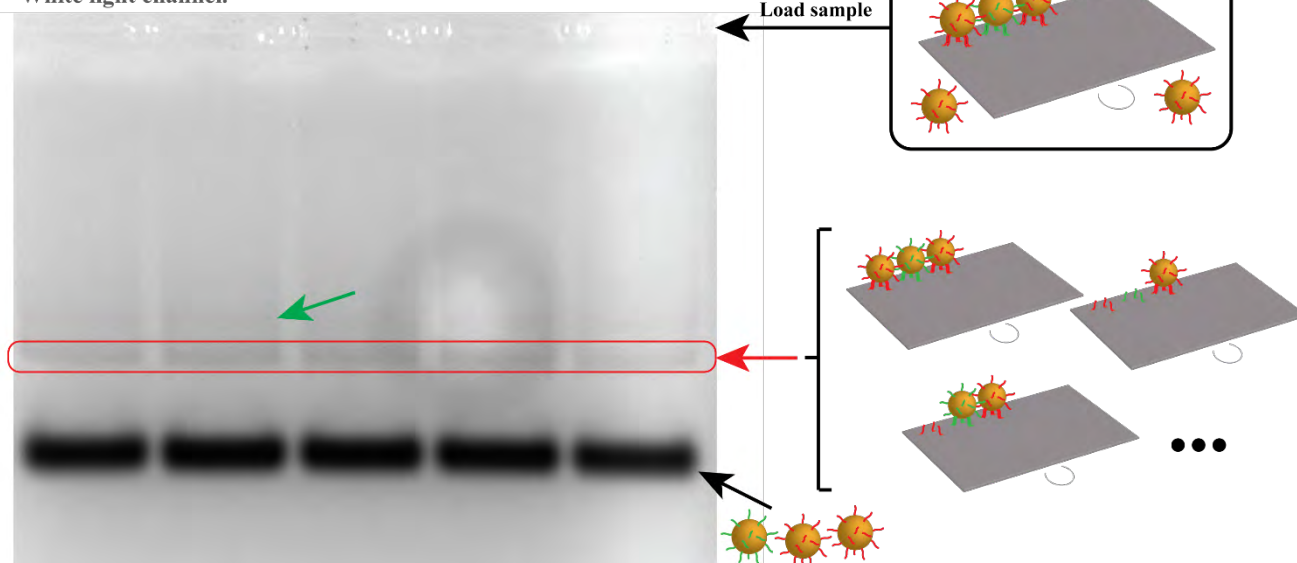
For all experiments with subsequent statistical analysis of the AFM/TEM images, samples were purified by agarose gel electrophoresis to remove the excess gold nanoparticle seeds added to increase the yield of the AuNP seed-origami assembly. Directly after the assembly step, the annealed AuNP-origami solution was mixed with 1/5 volume of glycerol/H<sub>2</sub>O (7:1 v/v) and loaded on a 0.5%-0.6% agarose gel in 1×TAMg buffer. The gel was then run at 80 V for 2 hrs in a cold room (4 °C) and imaged under the white light channel. A typical result is shown in **Fig. S6**. The second band from the bottom contains the target seed-origami assembly and thus was excised from the gel, cut into small pieces and transferred to a Freeze 'N Squeeze™ column. The column was frozen at -20 °C for 9 min and centrifuged at 13 kRPM for 9 min. The filtrate was carefully removed and the small dark-red pellet (containing the AuNP-origami assembly and sometimes small gel fragments) at the bottom of the tube was collected and re-dispersed in 1×HEPES buffer. The assemblies were then re-quantified on the plate reader after gel extraction based on the absorbance at 450 nm. Small gel fragments in the pellet significantly affect the quantification results and therefore need to be removed prior to plate reader quantification if present.

In a typical AGLO experiment (e.g. **Fig. 4b**), 0.065 pmol (regarding to the AuNP seed) seed-origami assembly was diluted with a solution containing 150  $\mu$ L 1×HEPESMg buffer, 5  $\mu$ L PVP360/H<sub>2</sub>O (5g/100mL) and 3.3  $\mu$ L 10×HEPESMg buffer in an Eppendorf tube. The diluted seed-origami solution was then chilled in an ice bath for 5 min, followed by adding 28  $\mu$ L gold source (0.06 g/100 mL HAuCl<sub>4</sub>/H<sub>2</sub>O solution) and carefully mixing with a pipette. The total volume of the experiment solution was kept at around 200  $\mu$ L and the HEPES concentration at 0.1 M (1×HEPESMg). The solution was then left in the ice bath for 3 hrs without disturbance, during which time the solution color will change from light pink to dark purple/blue. After the incubation, the Eppendorf was moved from the ice bath to room temperature and 2  $\mu$ L 0.1 M OEG/H<sub>2</sub>O solution was added and mixed by vortex. The following Lift-Off and washing steps are the same as discussed above. After washing and ultrasonication treatment, the crude product was directly imaged by TEM and analysed without further purification.

For the growth mechanism study (**Fig. 3**), 0.013 pmol seed-origami assembly and 1 – 6  $\mu$ L gold source were used but the seed-origami assembly concentration was kept around the same (total experimental volume around 43  $\mu$ L). In addition, TEM characterization was carried out after the incubation with OEG but before the Lift-Off and wash step to reserve non-merged Linear Trimer structures for the calculation and comparison of the merging percentage of the inter-particle gaps.

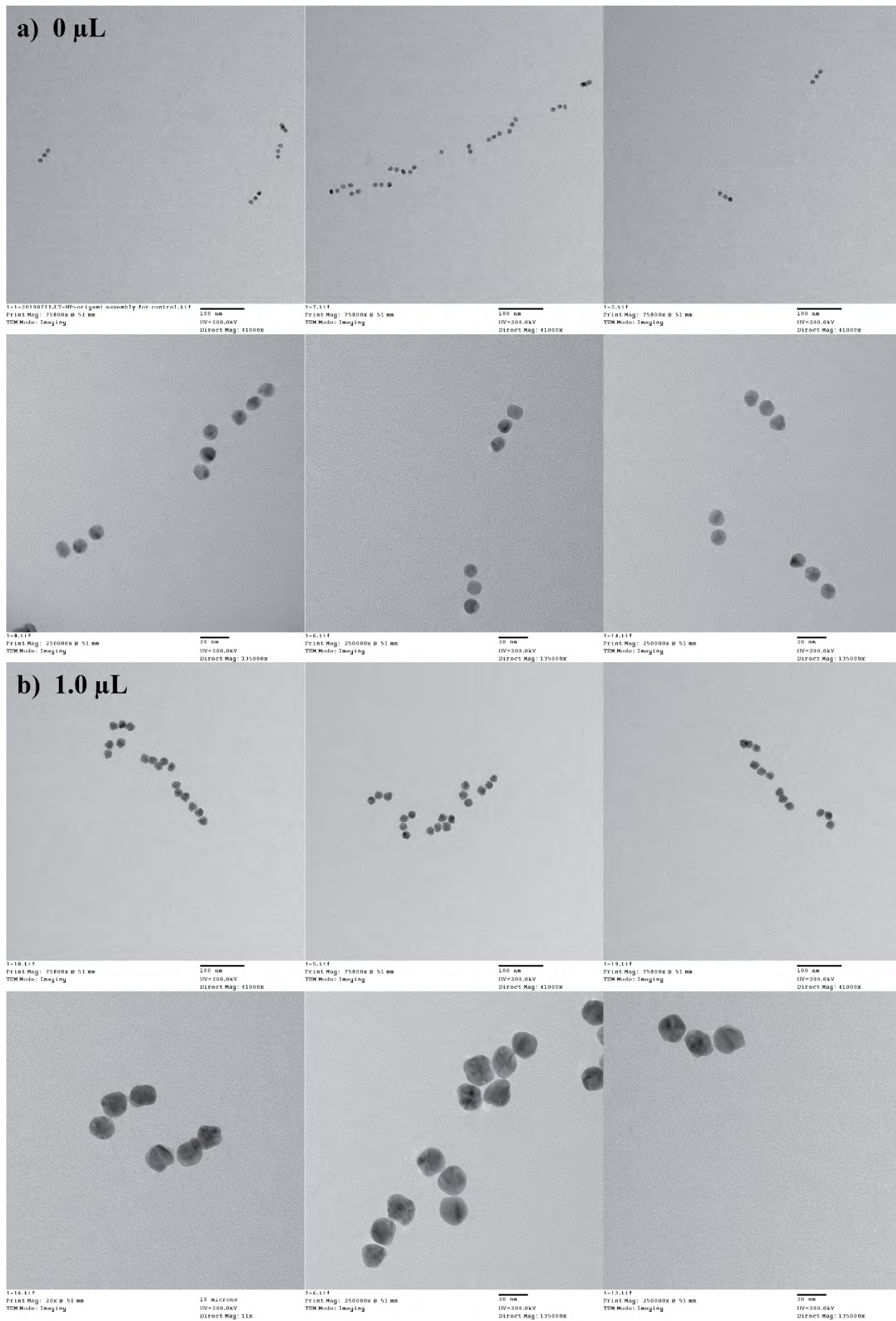
By changing the molarity of the seed-origami assembly or the gold source added, various final AuNP diameters can be reached. The merging yield generally increases with the final AuNP size; however, too much gold source applied at one time will result in random background nucleation. The final grown AuNP sizes in this study are controlled between 22 nm and 35 nm for better merging yield.

0.5-0.6% Agarose gel with 1×TAMg buffer, 80 V 2 hrs.  
White light channel.



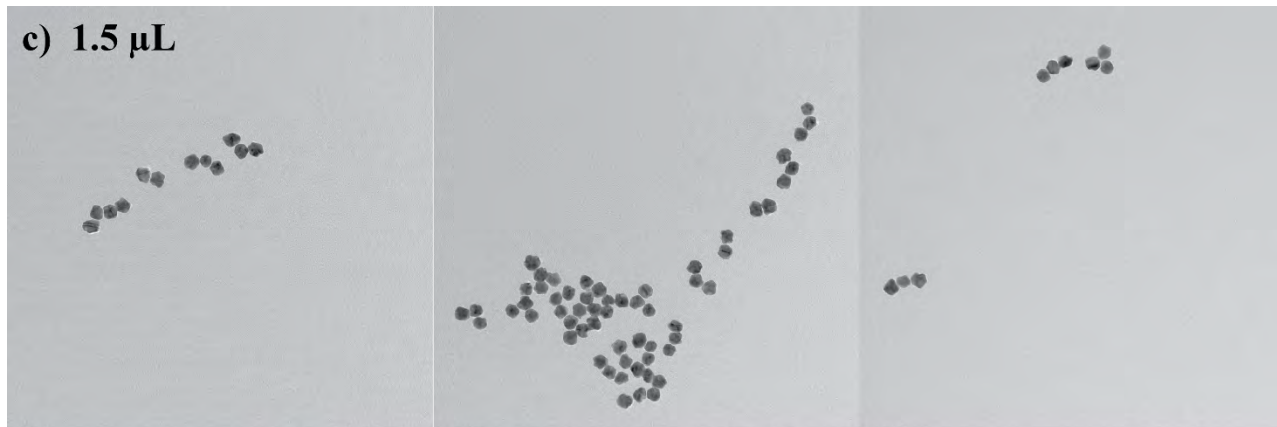
**Fig. S6.** A typical agarose gel electrophoresis purification of AuNP seed-origami assembly. The second band from bottom (red arrow) contains the target assembly of DNA origami templates with 1 to 3 AuNP seeds assembled to it. The gel cannot resolve these species, but after optimization of the binding conditions, the majority species should have three AuNP seeds assembled to the template. The faint band (green arrow) with lower mobility than the target assembly is a dimer of two DNA origamis with several AuNP seeds bound to them. The red band was subsequently excised, and the target assembly was extracted by a Freeze 'N Squeeze™ column.







c) 1.5  $\mu\text{L}$



2-6.tif  
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TEM Mode: Imaging

100 nm  
HV=200.0kV  
Direct Mag: 41000x

2-3.tif  
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100 nm  
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2-23.tif  
Print Mag: 75000x @ 51 mm  
TEM Mode: Imaging

100 nm  
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Direct Mag: 41000x



2-21.tif  
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TEM Mode: Imaging

20 nm  
HV=200.0kV  
Direct Mag: 135000x

2-15.tif  
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TEM Mode: Imaging

20 nm  
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Direct Mag: 135000x

2-4.tif  
Print Mag: 250000x @ 51 mm  
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20 nm  
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d) 2.0  $\mu\text{L}$



3-21.tif  
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TEM Mode: Imaging

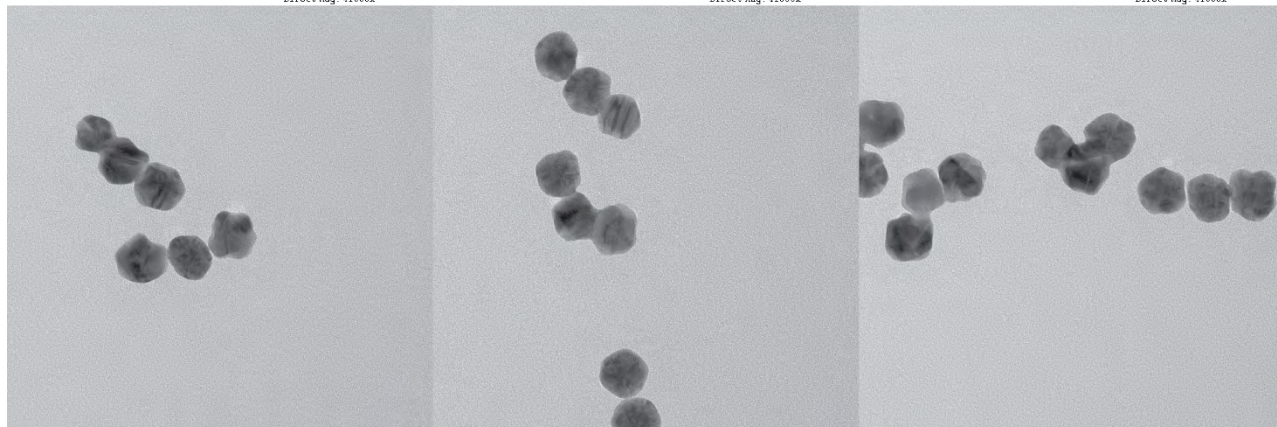
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3-5.tif  
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100 nm  
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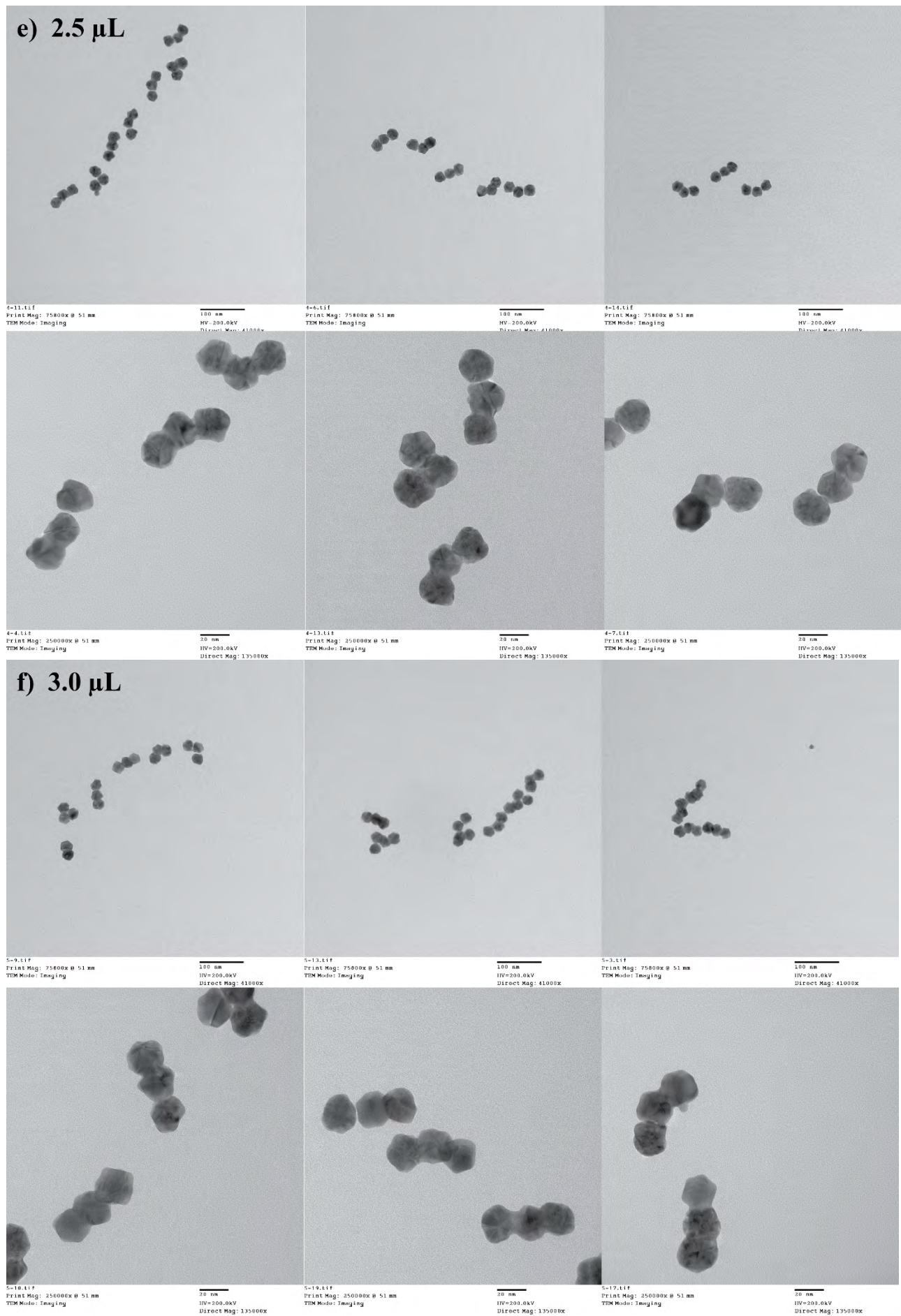
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20 nm  
HV=200.0kV  
Direct Mag: 135000x









6-10.11F  
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TEM Mode: Imaging

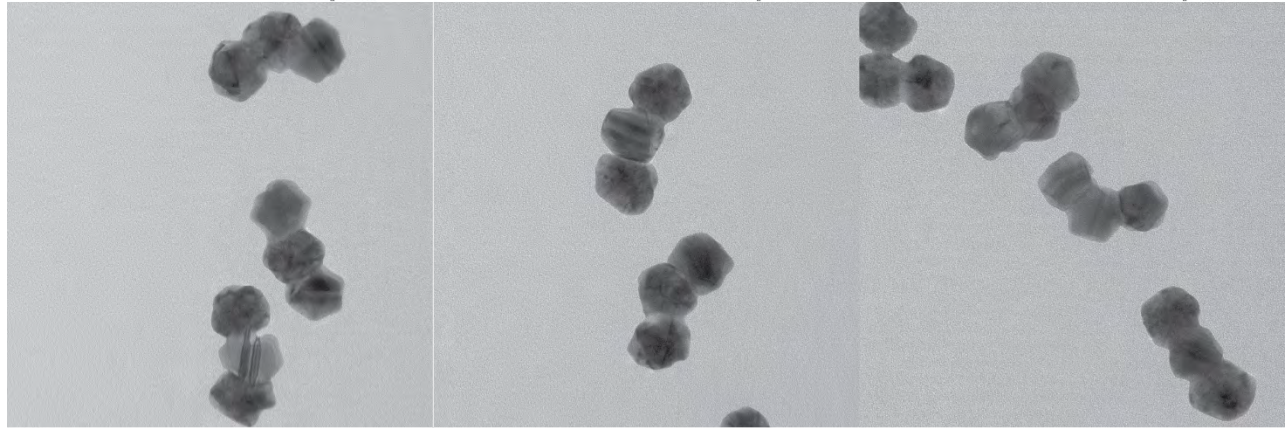
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6-6.11F  
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TEM Mode: Imaging

100 nm  
HV=200.0kV  
Direct Mag: 41000x

6-15.11F  
Print Mag: 75000x @ 51 mm  
TEM Mode: Imaging

100 nm  
HV=200.0kV  
Direct Mag: 41000x



6-16.11F  
Print Mag: 250000x @ 51 mm  
TEM Mode: Imaging

20 nm  
HV=200.0kV  
Direct Mag: 135000x

6-13.11F  
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TEM Mode: Imaging

20 nm  
HV=200.0kV  
Direct Mag: 135000x

6-3.11F  
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TEM Mode: Imaging

20 nm  
HV=200.0kV  
Direct Mag: 135000x



7-22.11F  
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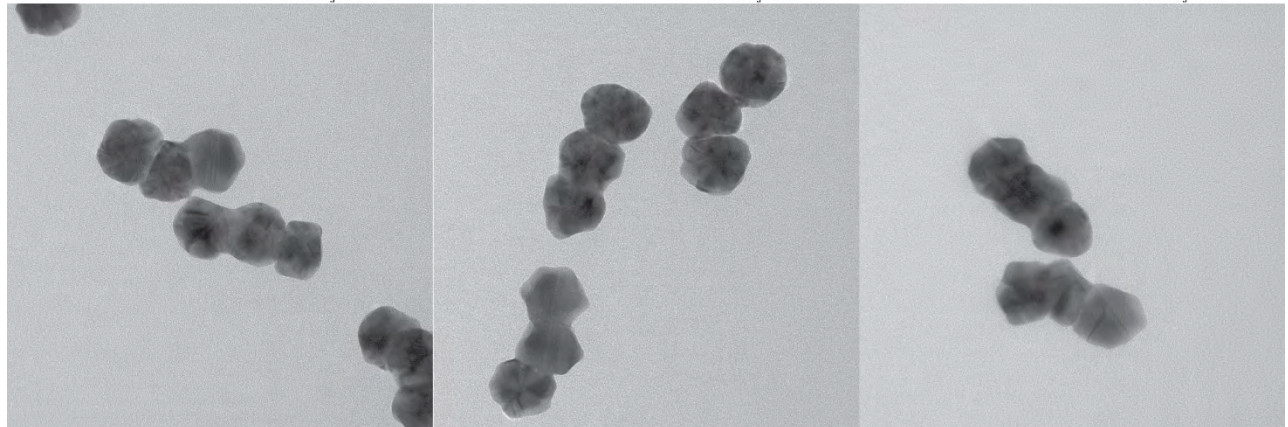
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7-35.11F  
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7-21.11F  
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TEM Mode: Imaging

100 nm  
HV=200.0kV  
Direct Mag: 41000x



7-5.11F  
Print Mag: 250000x @ 51 mm  
TEM Mode: Imaging

20 nm  
HV=200.0kV  
Direct Mag: 135000x

7-34.11F  
Print Mag: 250000x @ 51 mm  
TEM Mode: Imaging

20 nm  
HV=200.0kV  
Direct Mag: 135000x

7-10.11F  
Print Mag: 250000x @ 51 mm  
TEM Mode: Imaging

20 nm  
HV=200.0kV  
Direct Mag: 135000x

**Fig. S7.** Additional TEM images of samples in **Fig. 3** with **a)** 0  $\mu\text{L}$ , **b)** 1.0  $\mu\text{L}$ , **c)** 1.5  $\mu\text{L}$ , **d)** 2.0  $\mu\text{L}$ , **e)** 2.5  $\mu\text{L}$ , **f)** 3.0  $\mu\text{L}$ , **g)** 4.0  $\mu\text{L}$  and **h)** 6.0  $\mu\text{L}$  gold source (0.06%  $\text{HAuCl}_4$ ) applied in the AGLO process.

### 3. Fitting of the grown AuNP diameter (D) data

The volume of a AuNP during growth can be defined by a sphere volume function in equation (2), where  $V_0$  is the original volume of the AuNP seed,  $V'$  is the increase of the AuNP volume and  $D$  is the average diameter of the grown AuNP.

$$V = V' + V_0 = \frac{1}{6}\pi D^3 \quad (2)$$

Assuming that the increase of AuNP volume ( $V'$ ) is proportional to the amount of the gold source applied ( $S$ ) because all gold atoms deposited to the AuNP seed:

$$V' = a \times S \quad (3)$$

then equation (2) can be rewritten as:

$$D = \sqrt[3]{\frac{6a}{\pi}S + \frac{6V_0}{\pi}} = \sqrt[3]{\frac{6a}{\pi}} \times \sqrt[3]{S + \frac{V_0}{a}} \quad (4)$$

Equation (4) was used as the function for the fitting of the data in **Fig. 3d**. The fitting result is shown in equation (5) with an  $R^2$  value of 0.9975.

$$D = 18.026 \times \sqrt[3]{S + 0.253} \quad (5)$$

## S-VII. ImageJ assisted TEM analysis

### 1. To determine whether the inter-particle gaps are merged

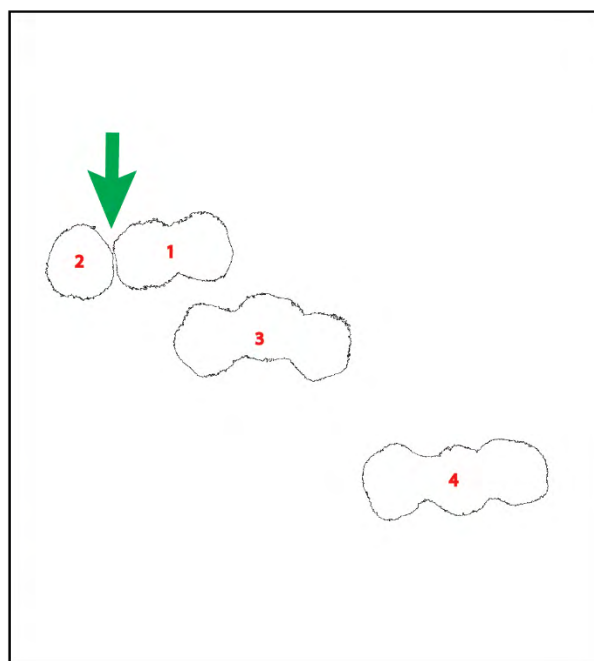
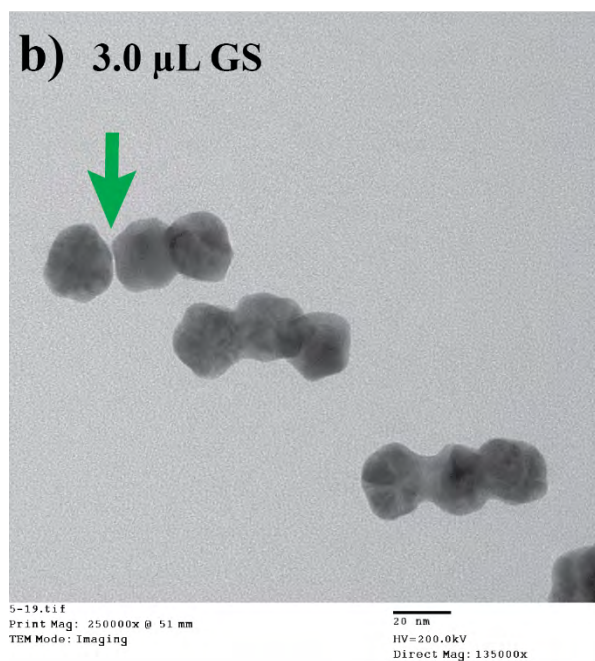
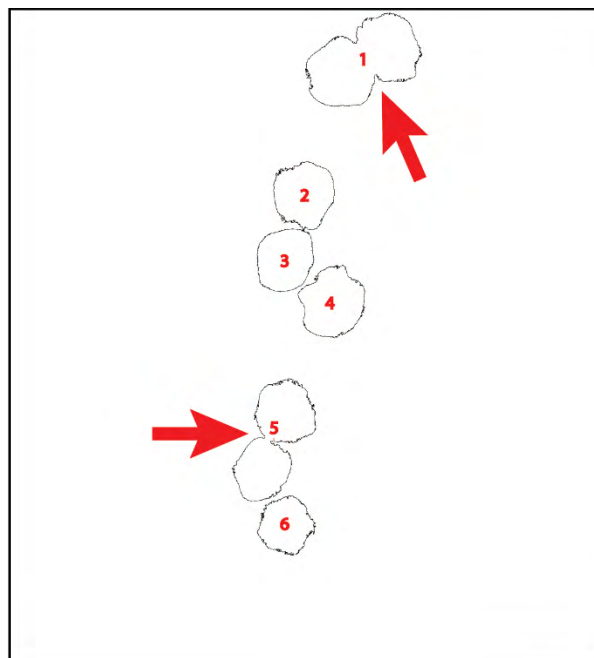
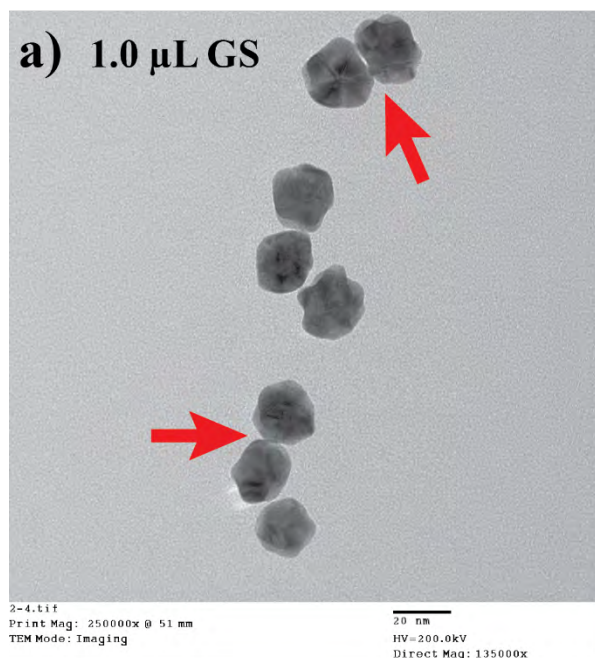
All AGLO products are passivated with OEG ligands after the gold growth, which endows the gold nanostructures with a negatively charged ligand shell. The repulsive ligand shell prevents the nanostructures from aggregating and ensures that non-merged AuNPs are generally not in contact even when they're closely packed due to the drying effect on the TEM grid.

The TEM image are first converted to a binary (black/white) image using the *Converted to Mask* option with the *Auto Threshold* default settings. *Analyze Particles* process were then carried out to determine how many particles were counted in the image (exclude particles on the image edge). In the result image (**Fig. S8**), merged gaps connect grown AuNPs and the whole structure is counted as one particle while non-merged gaps separate individual grown AuNPs.

### 2. Statistical analysis of the gold nanostructures in AGLO crude product

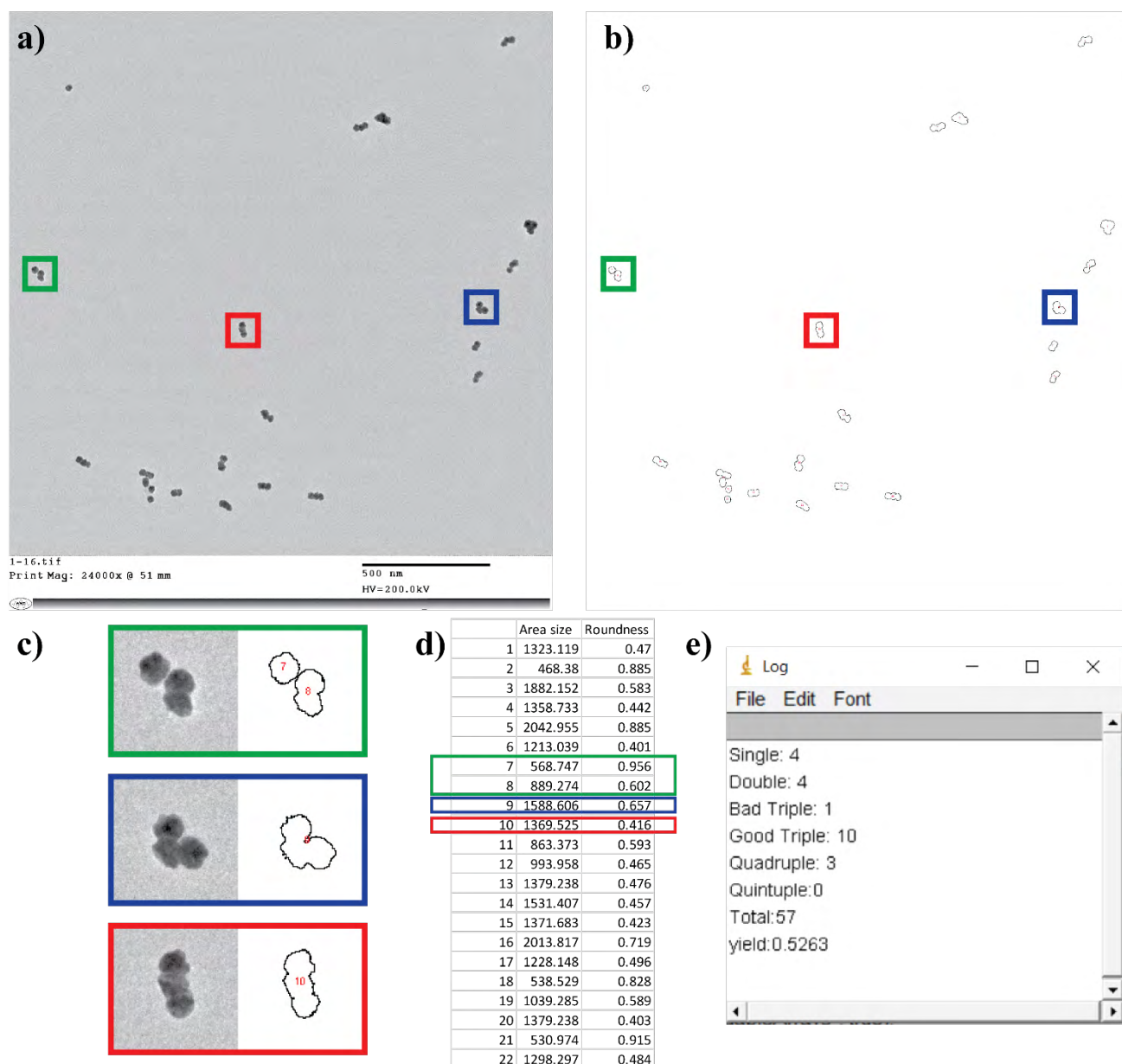
The analysis of the gold nanostructure distributions in the AGLO crude product was conducted using a custom FIJI<sup>6</sup> macro code, available on demand. After the *Analyze Particles* process described above, particle information (area size, roundness, aspect ratio, *etc.*) can be accessed and employed to differentiate the gold nanostructures. In our case, the particles in the TEM image are grouped based on their *Area Size* (monomer, dimer, trimer, tetramer, pentamer) and *Roundness* (Linear Trimer or others). The yield is calculated by the number of seeds in all Linear Trimer structures over the number of seeds in all structures (**Fig. S9**). Big AuNP aggregations (seeds number > 5 for the Linear Trimer experiments) were ignored in both AFM and TEM analysis. For each AGLO crude product, 20 to 25 TEM images were captured and analyzed for the gold nanostructure distribution result. The macro code allowed us to analyze hundreds of images in a short time and provided reliable statistical results (typically N>1000).

Note that structure percentages in the distribution diagrams are calculated differently than the yields. The structure percentages are calculated as the number of each merged gold nanostructure (not the number of seeds) over the total number of all merged structures counted.



**Fig. S8.** TEM image processing by the *ImageJ* to determine whether the inter-particle gaps are merged or not. **a)** an example of processed image from the sample with 1.0  $\mu$ L gold source in **Fig. 3** where only 6.5% gaps merged. Red arrows indicate the just merged gaps that are recognized by the program. **b)** an example of processed image from the sample with 3.0  $\mu$ L gold source in **Fig. 3** where 94.9% gaps merged. Green arrows indicate that the gap not merged can still be differentiated by the program.





**Fig. S9.** *ImageJ* based algorithm to count the number of simple merged structures in the TEM images of the AGLO product mixture. **a)** a sample TEM image with a few particles from the AGLO process of the Linear Trimer seed-origami assembly. **b)** software processed image with particle outlines and numbers. **c)** Zoomed-in images of some typical types of particles. Green: a not fully merged trimer recognized as one monomer and one dimer gold structure; Blue: a triangular trimer structure will be counted as a “Bad Triple” or “3 (others)” in the statistical results based on its Roundness value; Red: a fully merged Linear Trimer structure will be counted as a “Good Triple” or “3 (linear)” in the statistical results. **d)** Area size and Roundness data of corresponding particles will be measured by *ImageJ*. **e)** Algorithm output of this sample TEM image. Particles are grouped based on their Area Size (monomer, dimer, trimer, tetramer, pentamer) and Roundness (Linear Trimer or others). The yield is calculated by the number of seeds in all Linear Trimer structures over the number of seeds in all structures.

## S-VIII. Parameters affecting gold growth and merging

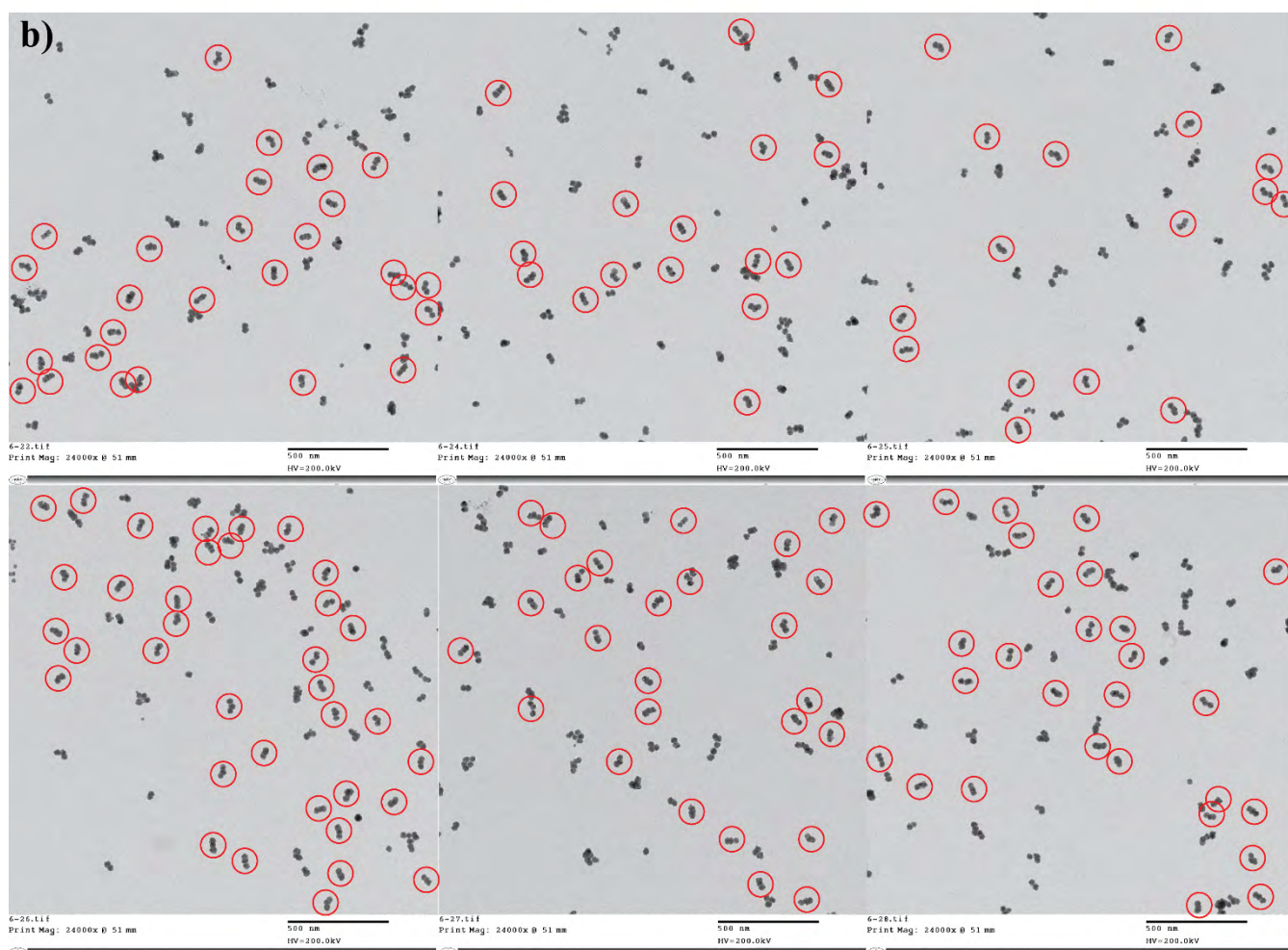
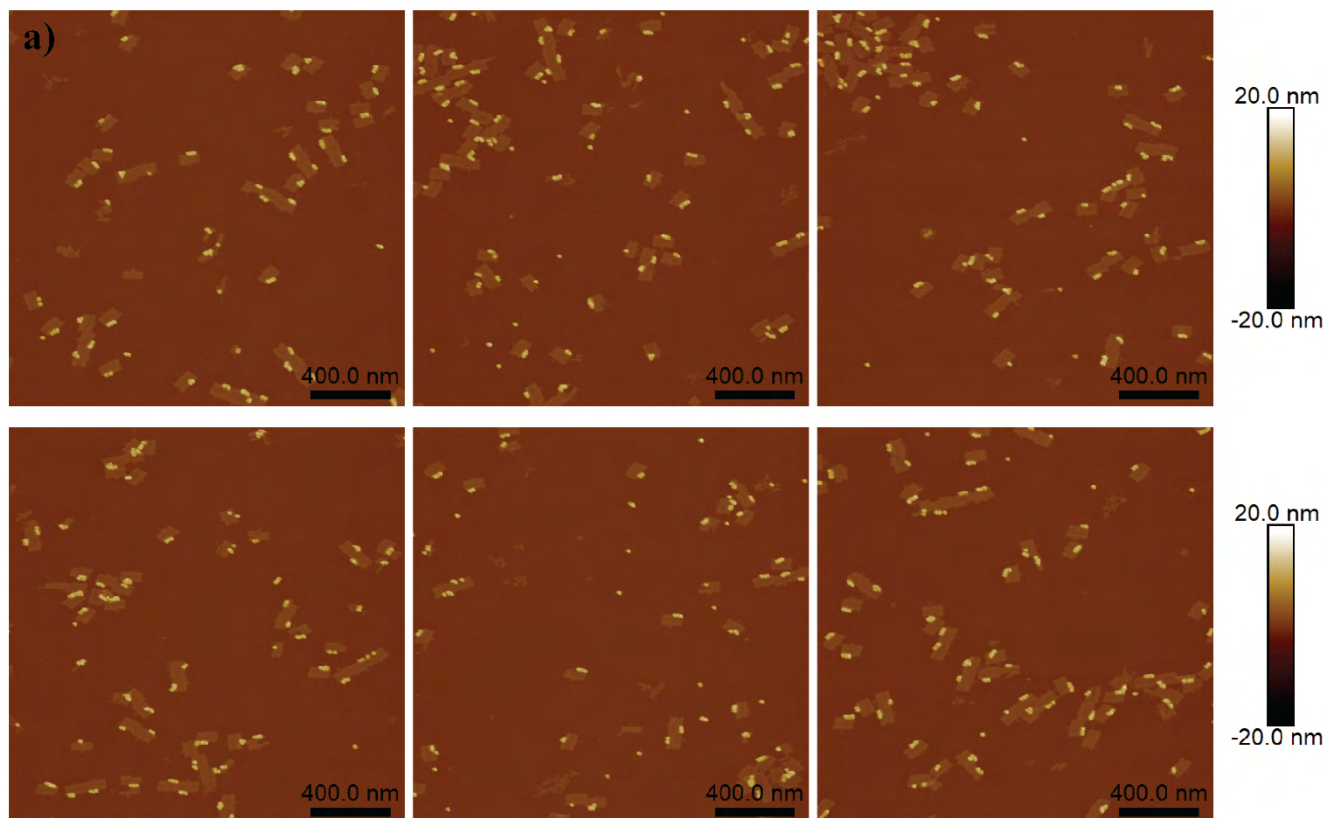
### 1. Surfactant

In the standard gold growth procedure discussed in S-VI-2, instead of 5  $\mu\text{L}$  PVP360/ $\text{H}_2\text{O}$  (5g/100mL), 1.25  $\mu\text{L}$  PVP10/ $\text{H}_2\text{O}$  (20g/100mL) or 1  $\mu\text{L}$  OEG/ $\text{H}_2\text{O}$  (0.1 M) were applied as the surfactant to study the influence of the surfactant on the gold growth and merging.

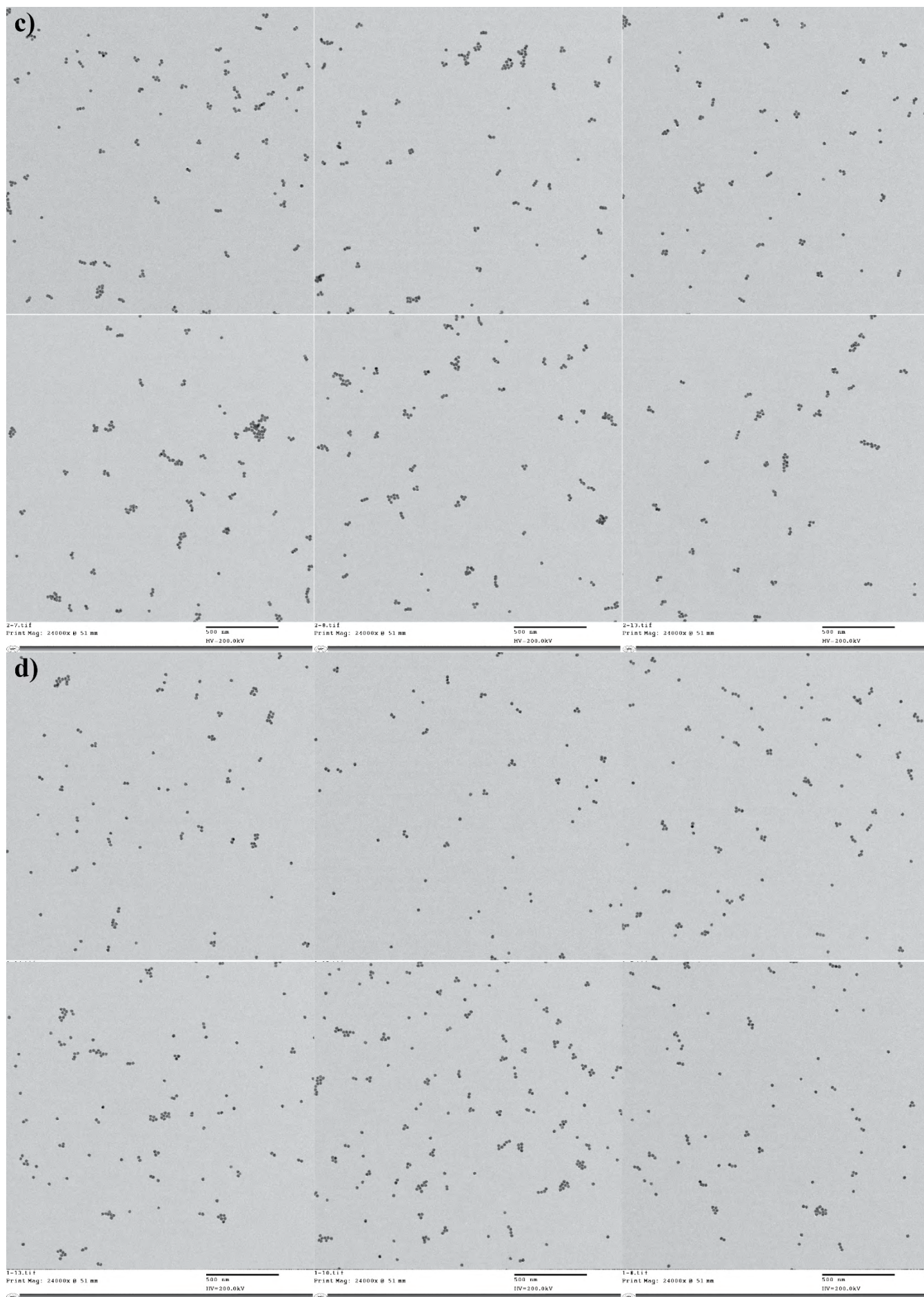
### 2. HEPES buffer aging under ambient light

In addition to the surfactant influence on the merging yield of the target structure in the AGLO method, we discovered that the aging of the HEPES buffer also has an impact on the yield. Among multiple experiments, the HEPES buffer that had been kept and used under ambient laboratory light at room temperature for more than 2 months, denoted as the Old Buffer, delivered higher yield of the merged Linear Trimer structure ( $45\pm 3\%$ ) than the freshly made HEPES buffer ( $25\pm 1\%$ ), denoted as the New Buffer. All other experimental conditions were kept the same. TEM images and the distribution of AuNP structures after the AGLO process with the Old and New Buffer (**Fig. S11**) indicated that with the New Buffer the AGLO product contained more grown AuNP monomers and random trimers (triangular trimers) than the Old Buffer, which in consequence led to a lower merged Linear Trimer yield. After thorough investigation of the Old and New Buffer, we precluded the suspicion that the HEPES molecule, buffer pH, salt concentration might have significantly changed during the aging of the buffer (**Fig. S12a-c**), which could cause alterations in the AGLO process. Intriguingly, HEPES buffer has been reported to generate  $\text{H}_2\text{O}_2$  under ambient fluorescent light illumination<sup>7-8</sup>, which we showed to be the case in our HEPES buffer system. As shown in **Fig. S12d**, the Old Buffer contained significantly higher amount of  $\text{H}_2\text{O}_2$  than the New Buffer.  $\text{H}_2\text{O}_2$  concentration were all measured in 10 $\times$ HEPESMg buffers. After keeping the Old Buffer in the dark at room temperature for a month, the  $\text{H}_2\text{O}_2$  slowly degenerated and reduced to around the same level of the New Buffer. To our surprise, such treated Old Buffer resulted in a low merged Linear Trimer yield, same as the New Buffer (**Fig. S12e**). Moreover, adding exogenous  $\text{H}_2\text{O}_2$  to the buffer with low  $\text{H}_2\text{O}_2$  concentrations before the gold growth step in AGLO recovered the merged target yield to some extent, although not as high as the original Old Buffer (**Fig. S12e, Fig. S13**). Given that  $\text{H}_2\text{O}_2$  is able to reduce the gold source to form AuNPs<sup>9-11</sup>, it is highly possible that it participated in the plating and gold growth process. Hence, we attribute most, but not all, of the different performances of the Old and New Buffer to the  $\text{H}_2\text{O}_2$  concentration. However, it is worth to point out that the  $\text{H}_2\text{O}_2$  colorimetry assay employed in this work is also responsive to other possible oxidants, such as organic hydroperoxides<sup>12-13</sup>, which are possible intermediates during the  $\text{H}_2\text{O}_2$  photo-generation process<sup>14</sup>. The detailed mechanism of how these molecules affect the merging of gold structures during the AGLO process remains elusive and is still under investigation.

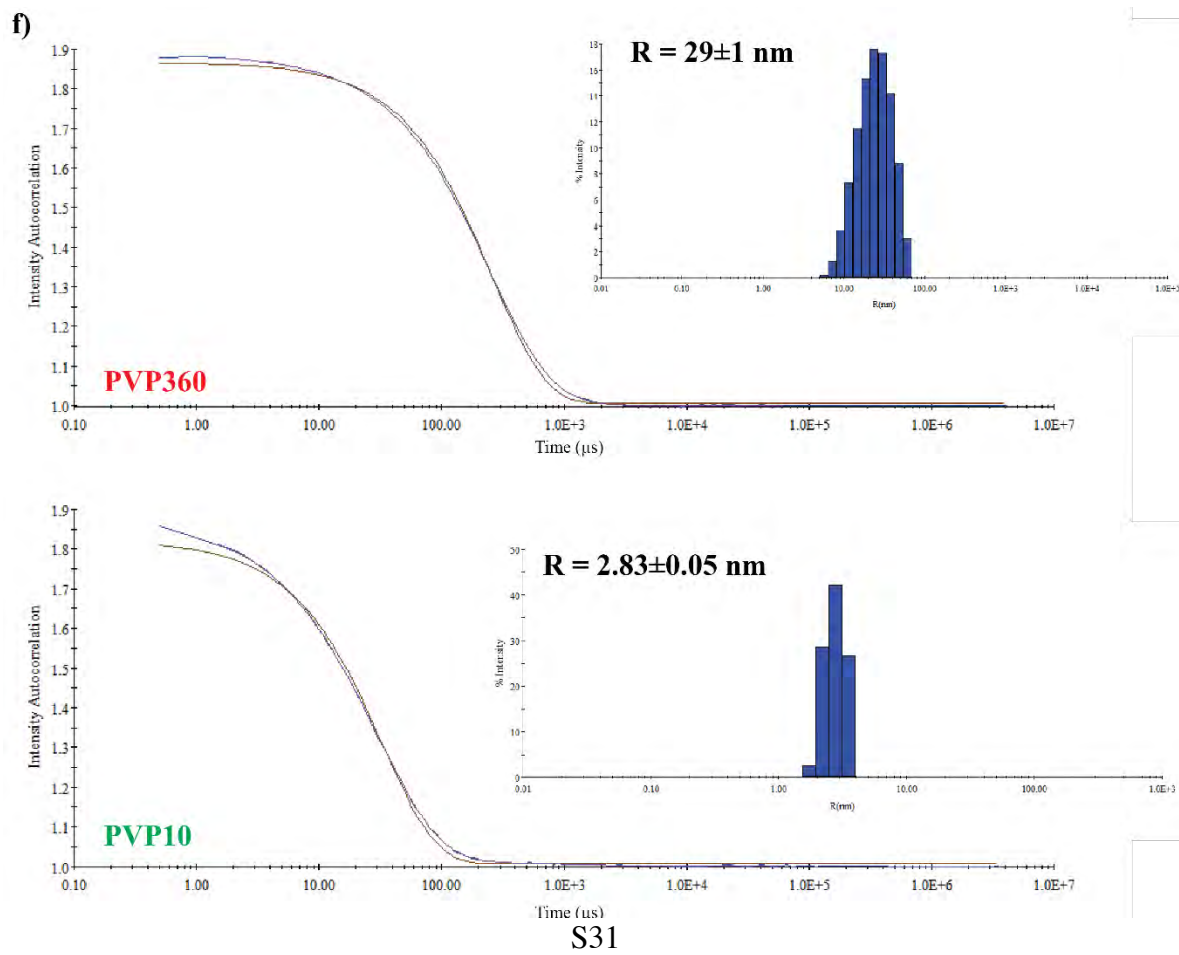
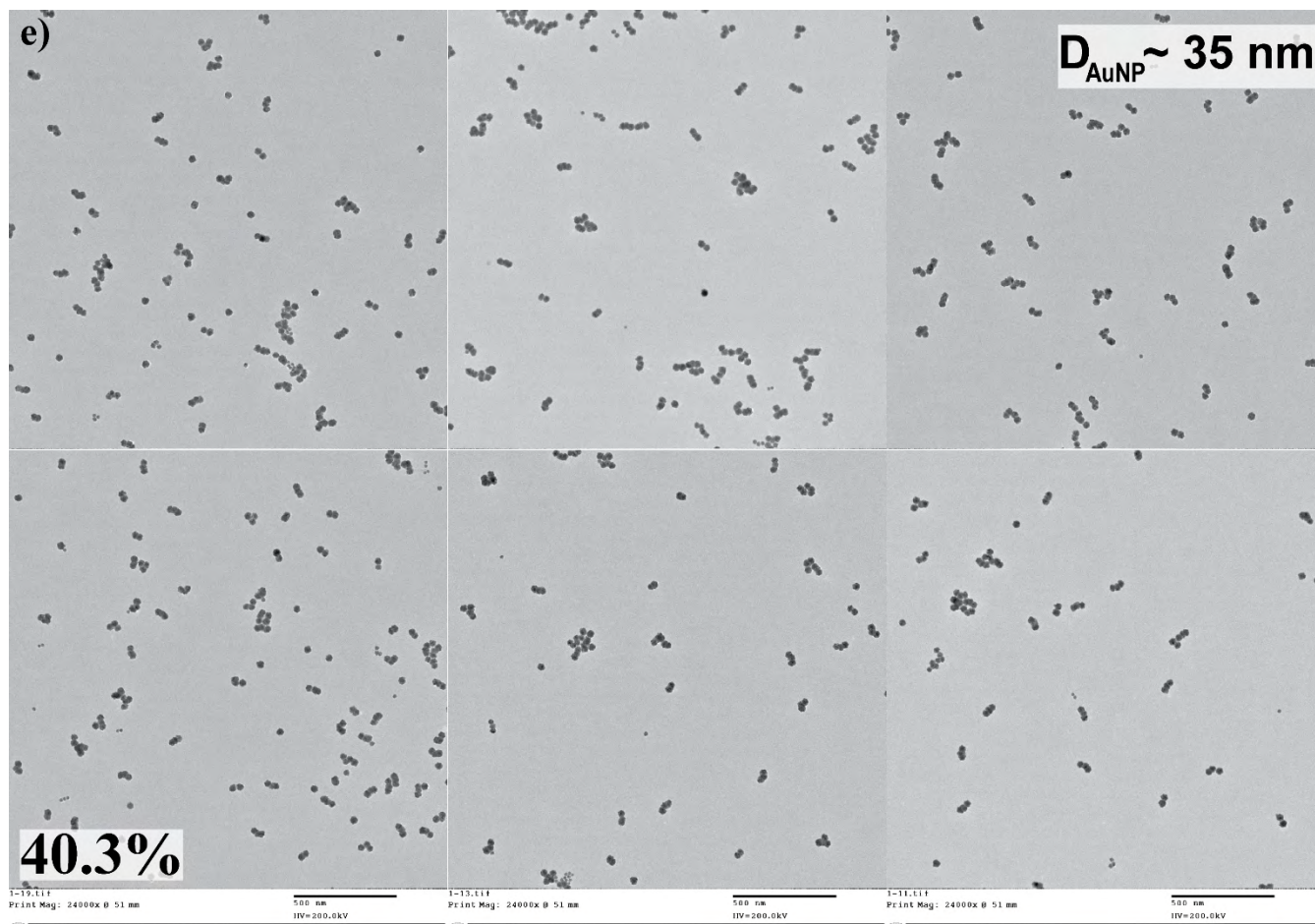


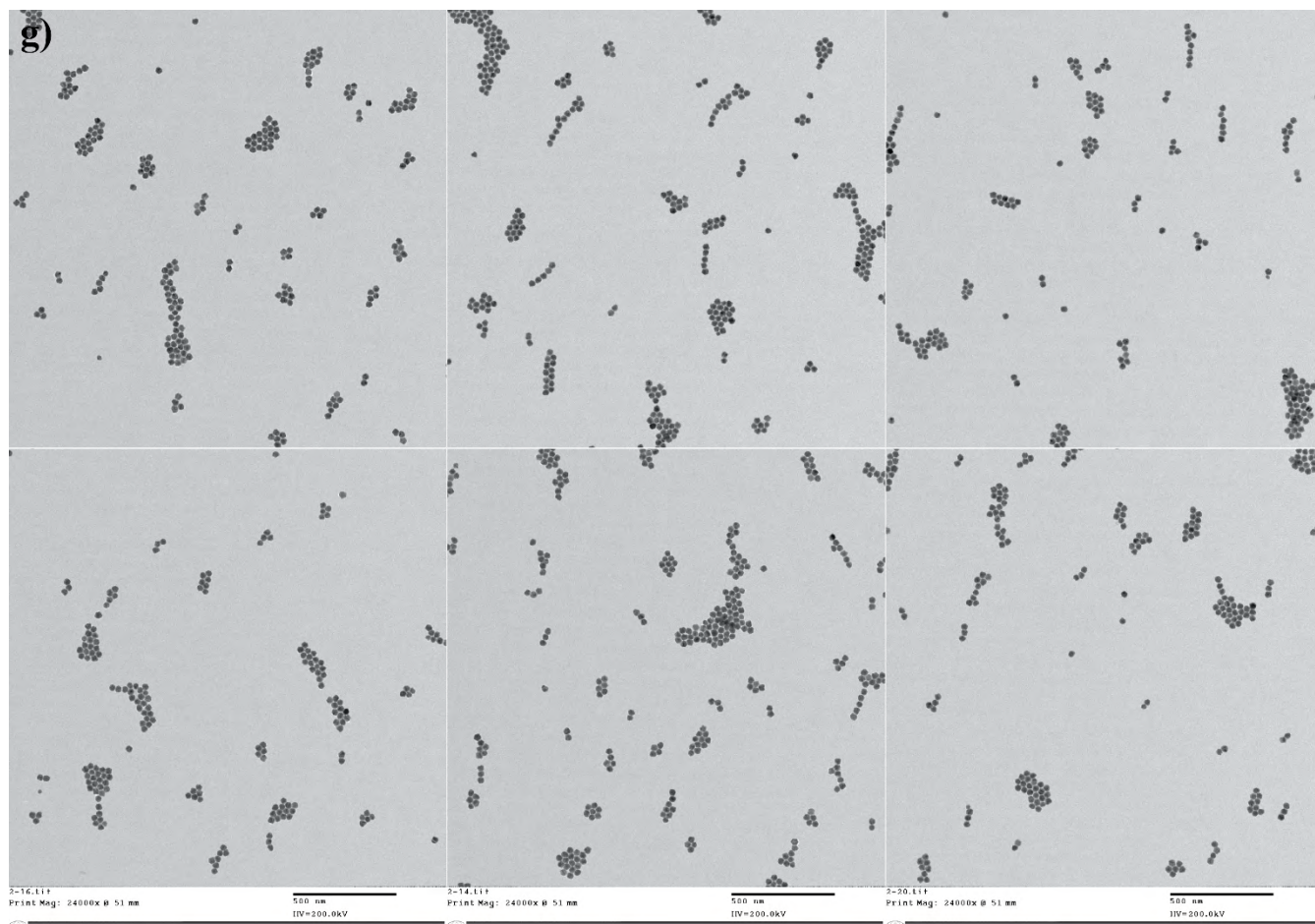








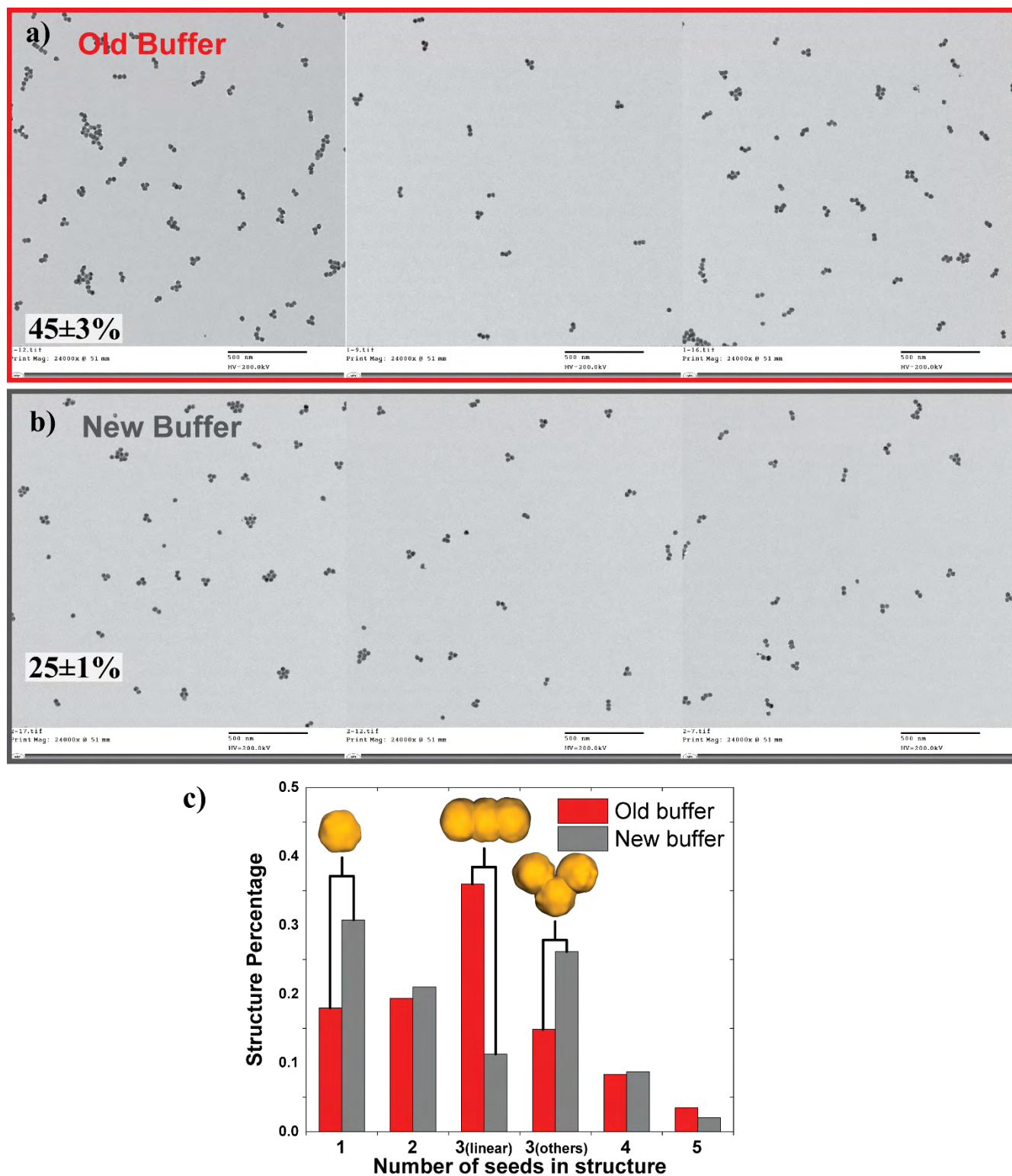




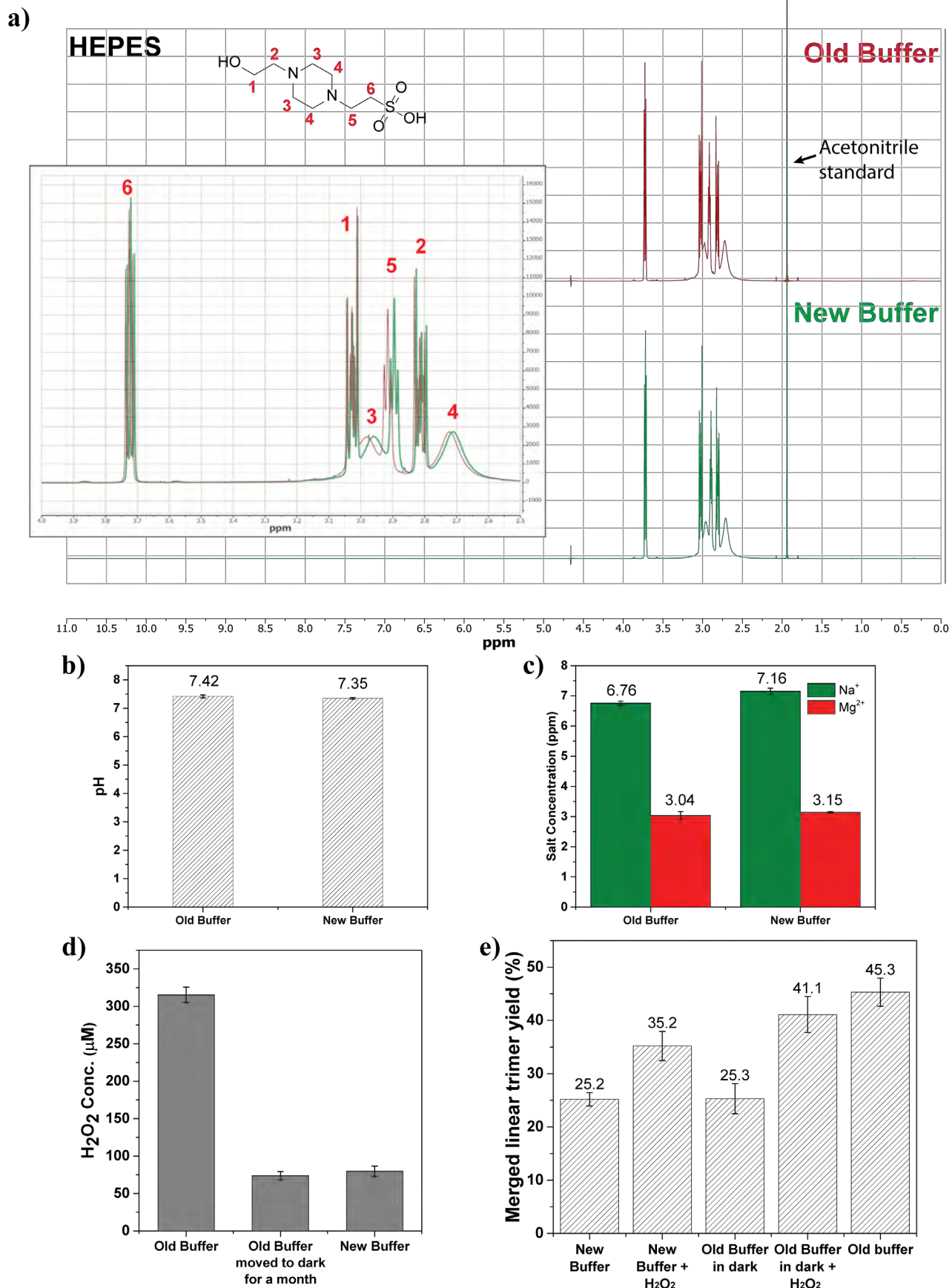
**Fig. S10.** **a)** Additional AFM images of the Linear Trimer AuNP seed-origami assembly after agarose gel purification. **b)** Additional TEM images of the AGLO product mixture with 28  $\mu\text{L}$  gold source and PVP360 as the surfactant. **c)** Additional TEM images of the AGLO product mixture with 10  $\mu\text{L}$  gold source and PVP360 as the surfactant. **d)** Additional TEM images of the AGLO product mixture with 10  $\mu\text{L}$  gold source and PVP10 as the surfactant. **e)** TEM images of the AGLO product mixture with excess amount of gold source (35  $\mu\text{L}$ ) and PVP10 as the surfactant. Applying excess gold source started to generate smaller AuNPs from random background nucleation. **f)** DLS analysis (correlation functions) of PVP360 and PVP10 polymers in HEPES buffer solution. Inserts: hydrodynamic radius distributions of the two molecules by DLS intensity. **g)** Additional TEM images of the AGLO product mixture with 28  $\mu\text{L}$  gold source and OEG as the surfactant.

With PVP10 the seed-origami structures are less likely to merge during the gold growth process, and subsequently more likely to disassemble during the denaturation and ultrasonication washes. However, when an excess amount of gold source (35  $\mu\text{L}$ ) was applied during the gold growth with PVP10, the yield of the merged Linear Trimer structure could reach the same level as with the PVP360 (**Supporting Information Fig. S10e**). In this case, the deformation of the origami template reaches the limit as the AuNP grows too large, the diminishing interparticle gap space can gradually force the PVP10 molecules out of the gap, allowing neighboring seeds to merge.





**Fig. S11.** TEM images of the AGLO product mixture with 20  $\mu\text{L}$  gold source, PVP360 as the surfactant and **a)** the Old Buffer or **b)** the New Buffer. **c)** The distribution of AuNP structures after the complete AGLO process with the Old Buffer (Red) or the New Buffer (Gray).



**Fig. S12.** a) D<sub>2</sub>O solvent suppression <sup>1</sup>H NMR spectra of the Old Buffer and the New buffer. 1×HEPES buffers were mixed with 20% volume of D<sub>2</sub>O and a drop of acetonitrile as the internal standard, and then

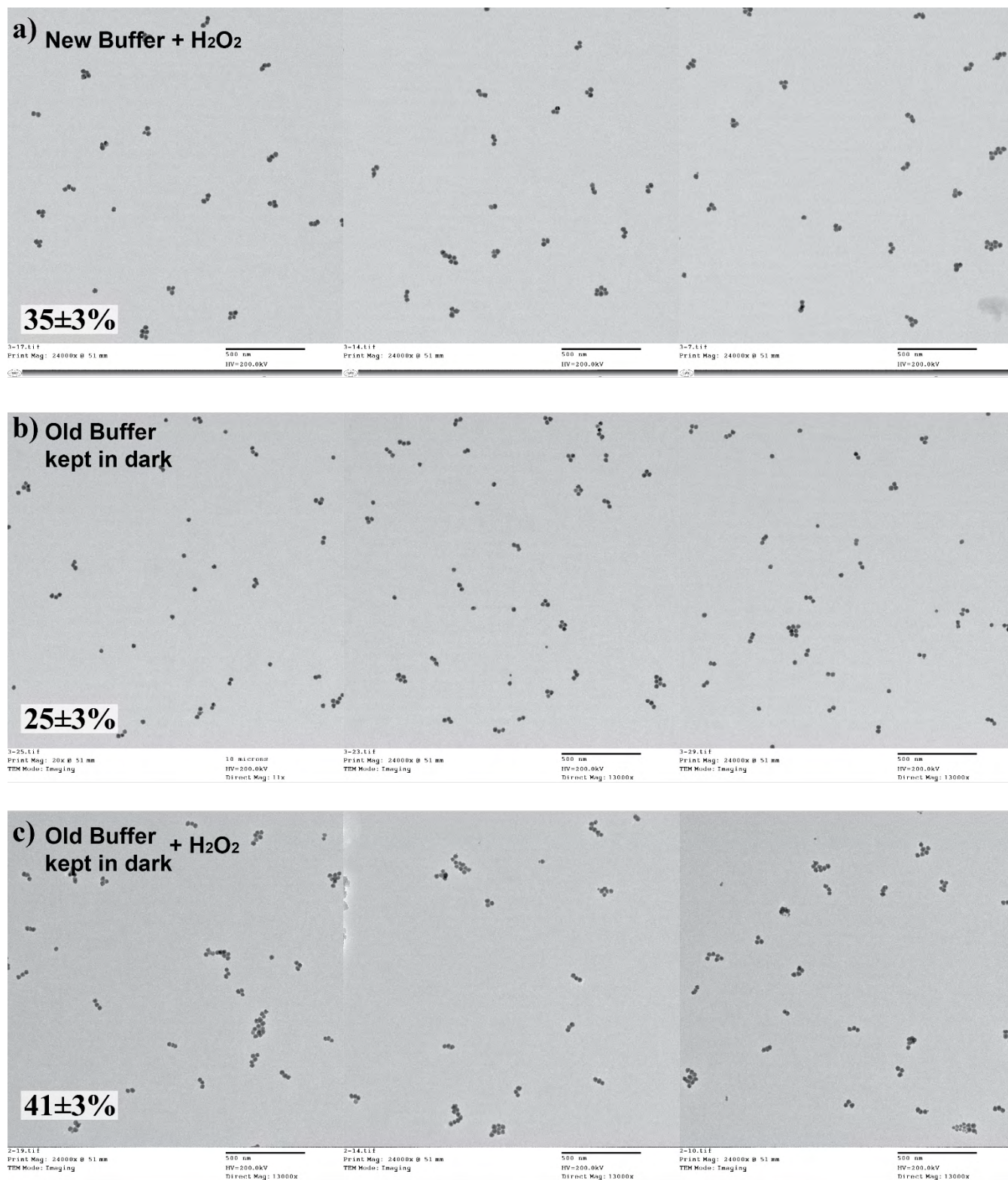
measured directly on a Bruker AVIIIHD 500 MHz NMR Spectrometer. The spectra are consistent with the literature<sup>15</sup> and no notable changes can be observed between the two buffers, indicating no detectable degradation of HEPES or generation of other molecules with <sup>1</sup>H NMR signals. The minor shift of peaks in the insert is probably caused by slight differences in protonation. **b)** pH measurement of the Old and the New 10×HEPES buffer. **c)** Na<sup>+</sup> and Mg<sup>2+</sup> concentrations of the Old Buffer and the New buffer measured by atomic absorption spectroscopy (AAS). 1×HEPES buffers were diluted 100 times with Milli-Q water and measured by a Perkin Elmer AAnalyst 800 atomic absorption spectrometer. The Na<sup>+</sup> difference originates from the NaOH amount used to adjust the pH when making the HEPES buffers. **d)** H<sub>2</sub>O<sub>2</sub> concentrations of an Old Buffer, an Old Buffer kept in dark for a month and a New Buffer. Measurements are carried out in triplicates on different days on the 10×HEPESMg buffers. **i)** AGLO merged Linear Trimer yield with New Buffer, New Buffer with exogenous H<sub>2</sub>O<sub>2</sub> upon experiments, Old Buffer kept in dark for a month, Old Buffer kept in dark for a month with exogenous H<sub>2</sub>O<sub>2</sub> upon experiments, and Old Buffer. All experiments were carried out in triplicates.

### 3. H<sub>2</sub>O<sub>2</sub> colorimetry assay

The H<sub>2</sub>O<sub>2</sub> concentrations of the 10×HEPESMg buffers were measured by an adapted ferric-xylenol orange assay<sup>12-13</sup>. The assay solution contains 10 μL Xylenol Orange (1.5 mM), 10 μL (NH<sub>4</sub>)<sub>2</sub>Fe(SO<sub>4</sub>)<sub>2</sub> (1.5 mM) and 70 μL H<sub>2</sub>SO<sub>4</sub> (50 mM), with a total volume of 90 μL. For the standard curve, 10 μL H<sub>2</sub>O (blank) or 10 μL H<sub>2</sub>O<sub>2</sub> (50, 100, 200, 400, 600, 800 μM) solutions were each mixed with 90 μL assay solution and incubated for 10 mins in a 96-well plate at room temperature. Then the absorbance at 550 nm of the standard samples were measured on a plate reader. The standard experiments were carried out in triplicates and a standard curve was plotted and fitted. Next, 10 μL of various 10×HEPESMg buffers were mixed with 90 μL assay solution and their absorbance at 550 nm was measured in the same way. Their H<sub>2</sub>O<sub>2</sub> concentrations were directly calculated from the standard curve.

For Old and New buffer experiments with exogenous H<sub>2</sub>O<sub>2</sub>, 510 nmol H<sub>2</sub>O<sub>2</sub> was added to the buffer solution with PVP360 and mixed well prior to the addition of the AuNP seed-origami assembly and the gold source in the standard procedure discussed in S-VI-2.





**Fig. S13.** TEM images of the AGLO product mixture with 20  $\mu$ L gold source, PVP360 as the surfactant and **a)** the New Buffer with added exogenous H<sub>2</sub>O<sub>2</sub>, **b)** the Old Buffer after being kept in dark for a month or **c)** the Old Buffer after being kept in dark for a month with added exogenous H<sub>2</sub>O<sub>2</sub>.



## S-IX. Various complex gold nanostructures constructed by AGLO

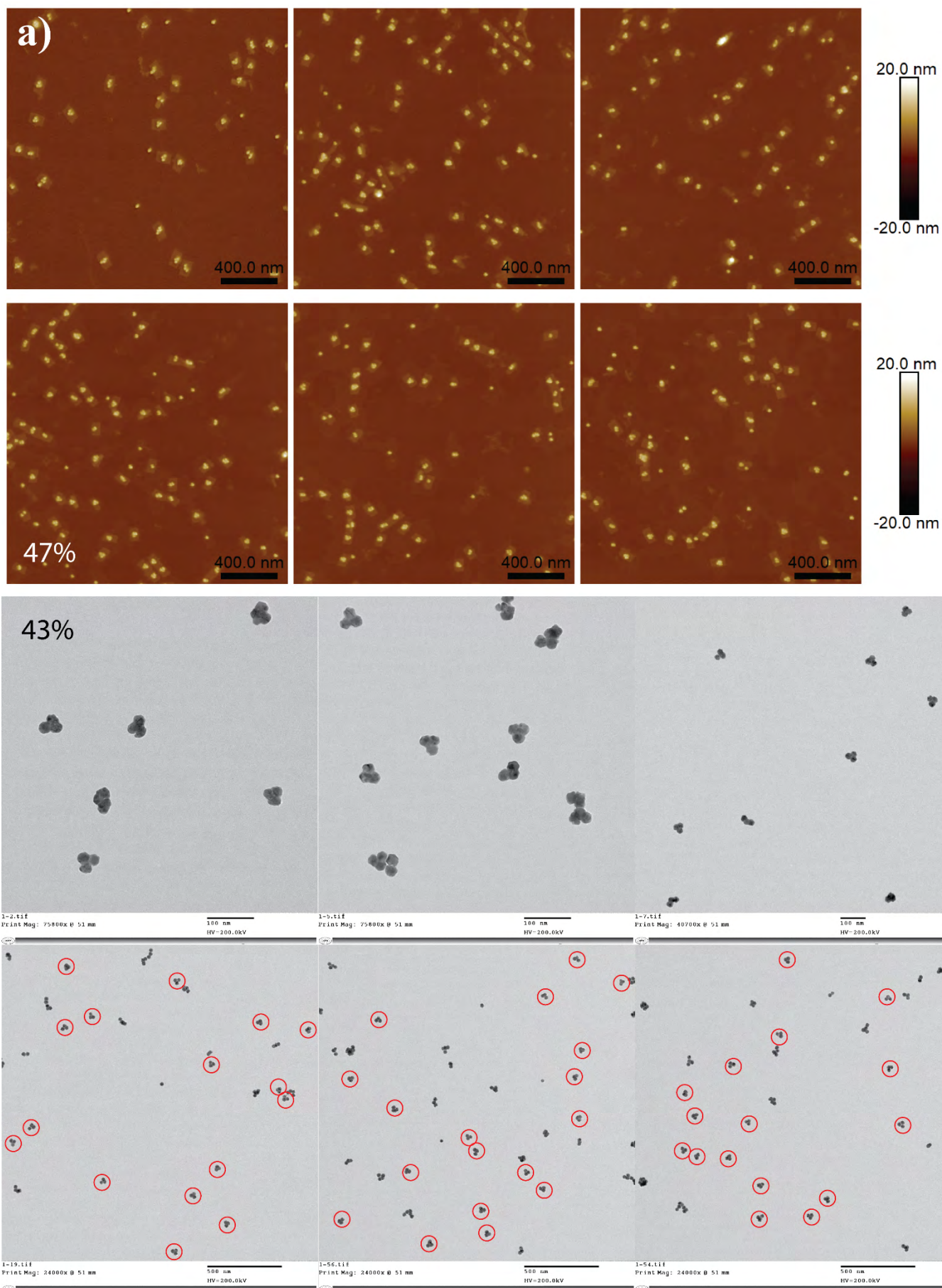
Modified staple strands used in each DNA origami template for the corresponding gold nanostructures were listed in **Table S3**. The rest of the staple strands were from the original origami design. Modified staple strand sequences can be found in **Table S2**.

**Table S3. Modified staple strands applied in each DNA origami design.**

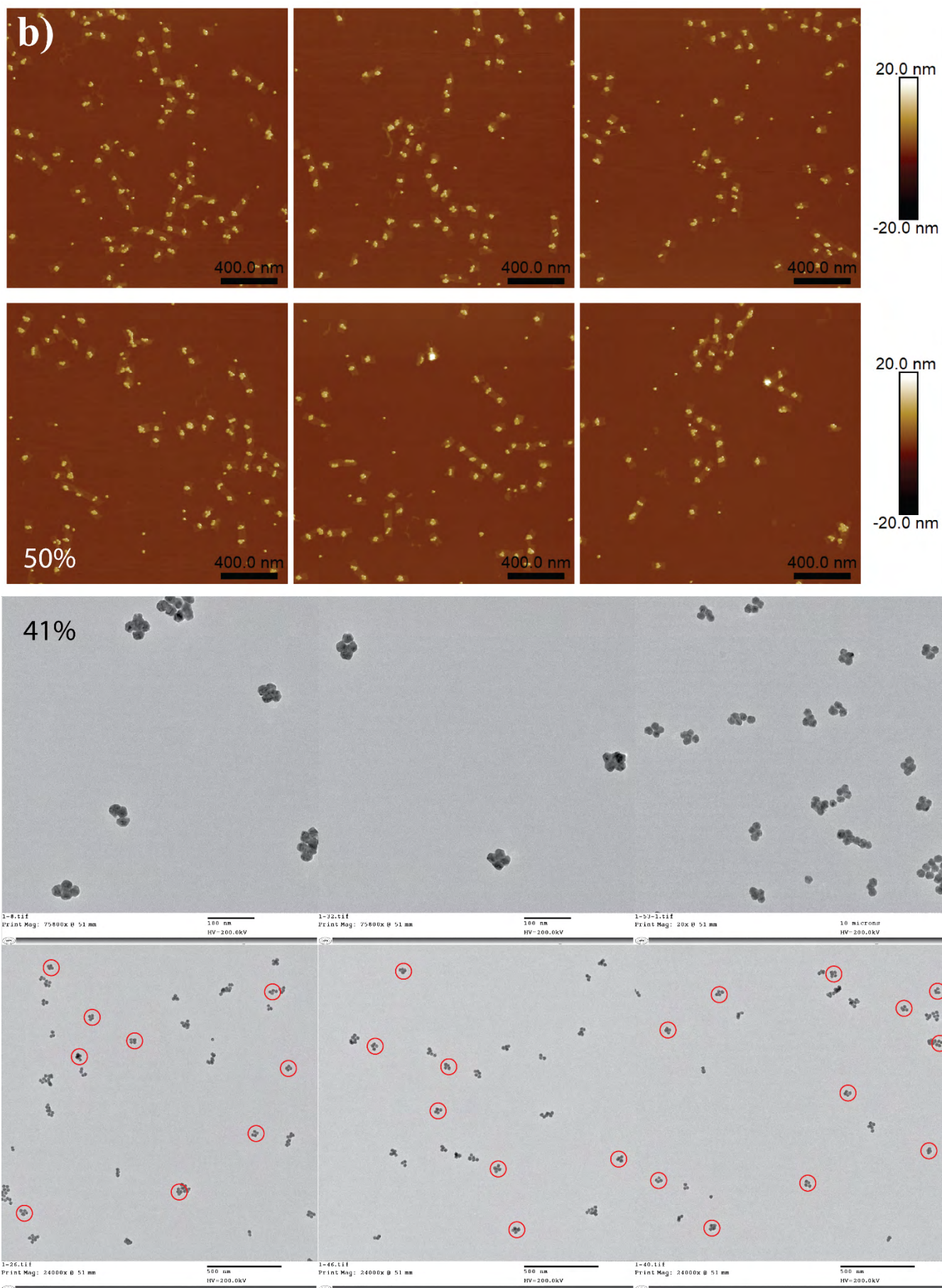
Gold nanostructure	Modified staple strands
Linear Trimer	2 BS1', 126 BS1', 128 BS1'; 56 BS1', 58 BS1', 77 BS1'; 29 BS2', 31 BS2', 34 BS2'.
Triangular Trimer	9 BS2', 121 BS2', 123 BS2'; 122 BS1', 140 BS1', 142 BS1'; 17 BS1', 19 BS1', 39 BS1'.
Tetramer Cluster	9 BS2', 121 BS2', 123 BS2'; 18 BS2', 114 BS2', 116 BS2'; 122 BS1', 140 BS1', 142 BS1'; 17 BS1', 19 BS1', 39 BS1'.
Y-shape Tetramer	2 BS1', 126 BS1', 128 BS1'; 9 BS2', 121 BS2', 123 BS2'; 122 BS1', 140 BS1', 142 BS1'; 17 BS1', 19 BS1', 39 BS1'.
L-Shape pentamer	2 BS1', 126 BS1', 128 BS1'; 68 BS1', 70 BS1', 89 BS1'; 15 BS1', 16 BS1', 116 BS1'; 41 BS2', 43 BS2', 46 BS2'; 9 BS2', 121 BS2', 123 BS2'.
Box-shape octamer	2 BS1', 126 BS1', 128 BS1'; 56 BS1', 58 BS1', 77 BS1'; 68 BS1', 70 BS1', 89 BS1'; 15 BS1', 16 BS1', 116 BS1'; 29 BS2', 31 BS2', 34 BS2'; 64 BS2', 83 BS2', 85 BS2'; 41 BS2', 43 BS2', 46 BS2'; 9 BS2', 121 BS2', 123 BS2'.

As the complexity of the target gold nanostructure increased, the overall yields of these target structures decreased from 43% of the Triangular Trimer to 18% of the Box-shape Octamer. However, the target structure transformation percentages of all these structures were above 80% and up to 92%, which indicated that the gold growth and merging process was highly efficient, and the lower AGLO yields of more complex structures were primarily due to their low seed-origami assembly yields before the gold growth step. It is worth pointing out that we did not optimize the distance of the origami binding sites in these designs individually, which could be carried out to improve the yield of the correctly assembled seed-origami structures. Additionally, the single layer 2D origami template is most likely too flexible to host large AuNP assemblies, as deformed and defective origami templates were often observed when more than 4 AuNP seeds were assembled on the template. It is even more so when AuNP seeds are designed to bind to sites close to the template edges (**Fig. S15**). The template flexibility and deformation also explain the decrease of the seed-origami assembly yield from 50% (Tetramer Cluster) to 31% (Y-shape Tetramer) which have the same number of seeds (**Fig. S14f**, green boxes); because the extended Y-shape is more subject to deformations caused by the template flexibility than the cluster shape. Nonetheless, it is worth to mention that previously in the literature, most studies do not provide the overall

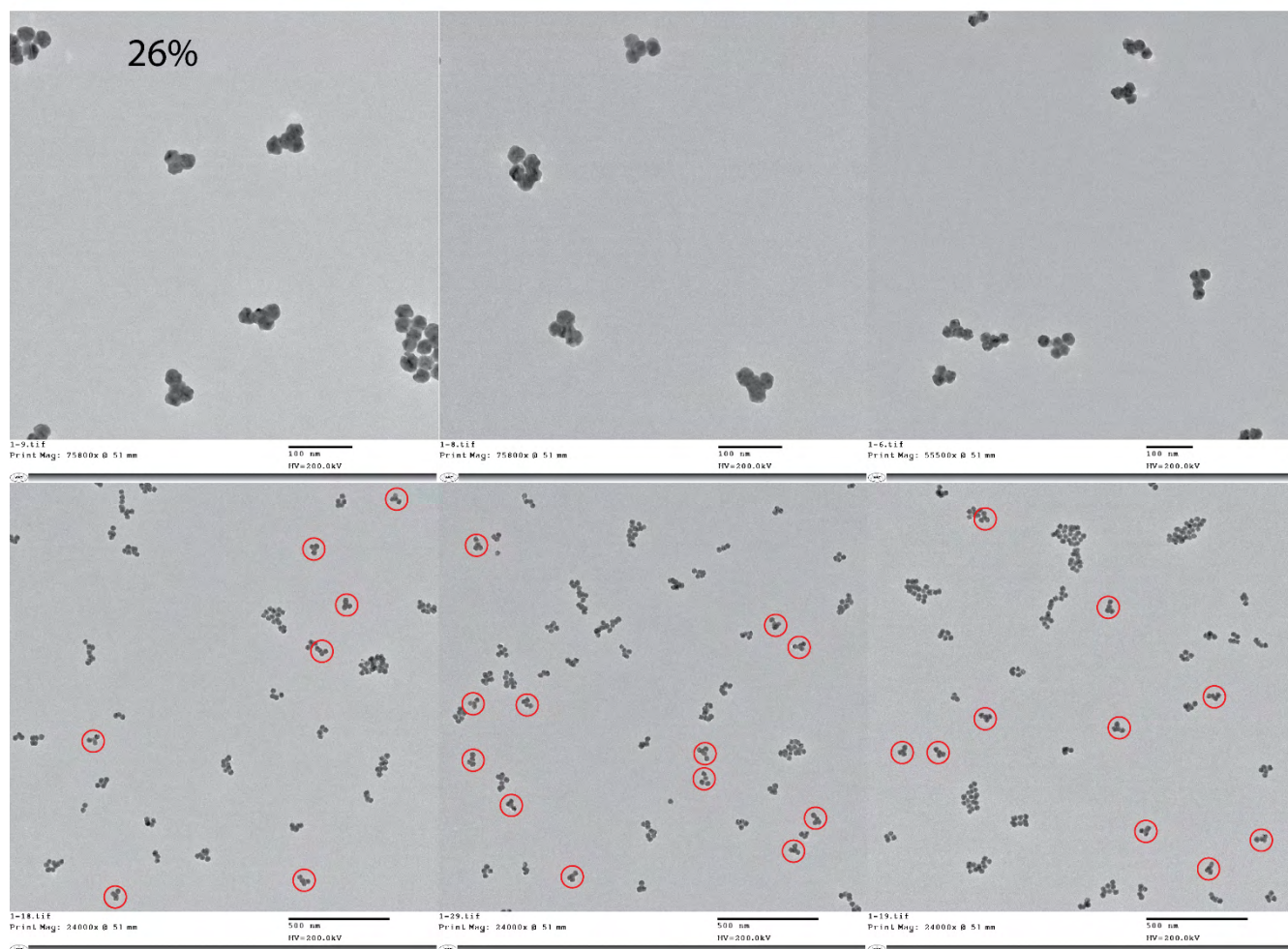
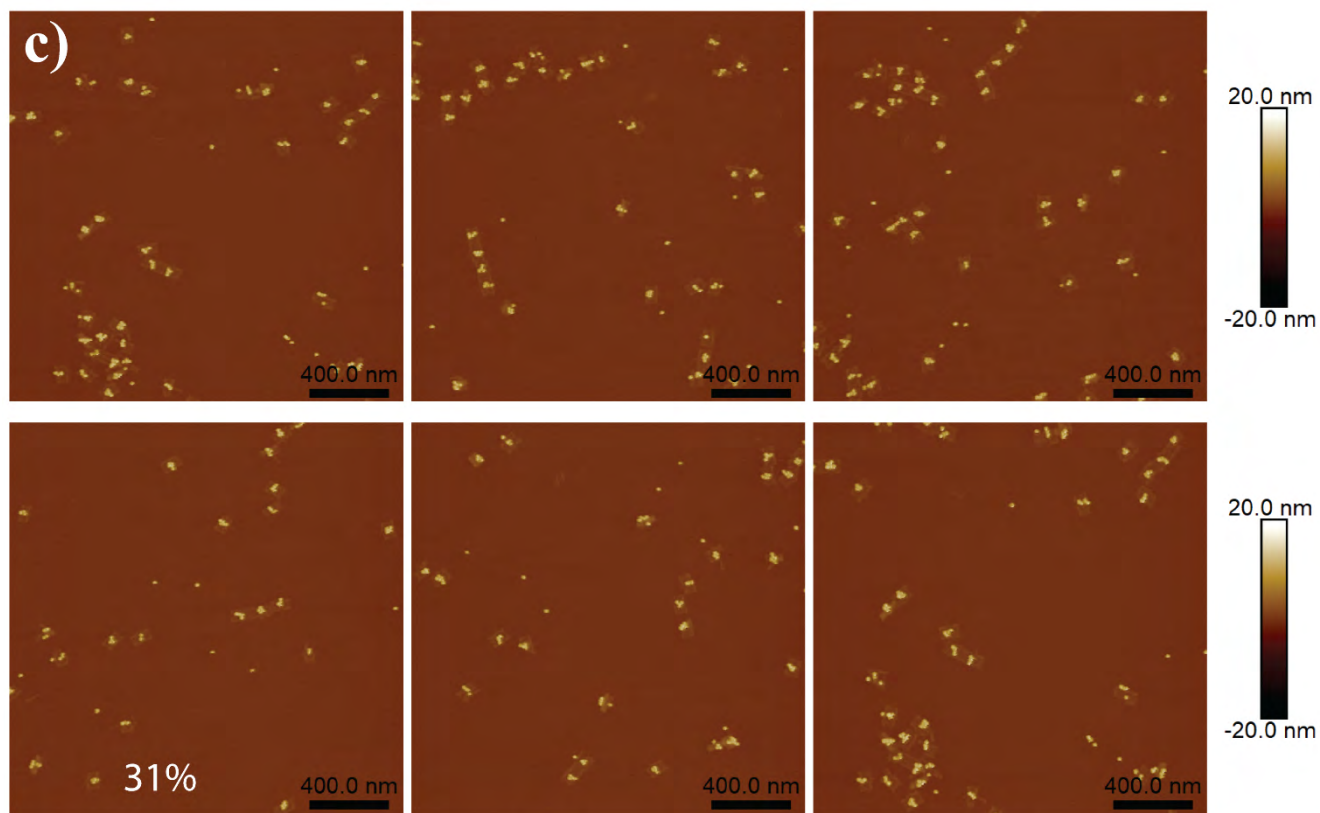
yield of synthesized structures after the metal growth due to the non-uniform structure morphologies and the uncontrollable background aggregation (ref. 45 - ref. 48). Only a more recent study reported similar yields for the trimer, tetramer and hexamer structures (ref. 49). However, the structures were grown on a flat surface without disturbance and imaged right after, thus the actual yield of fully merged robust standalone structures is not clear.



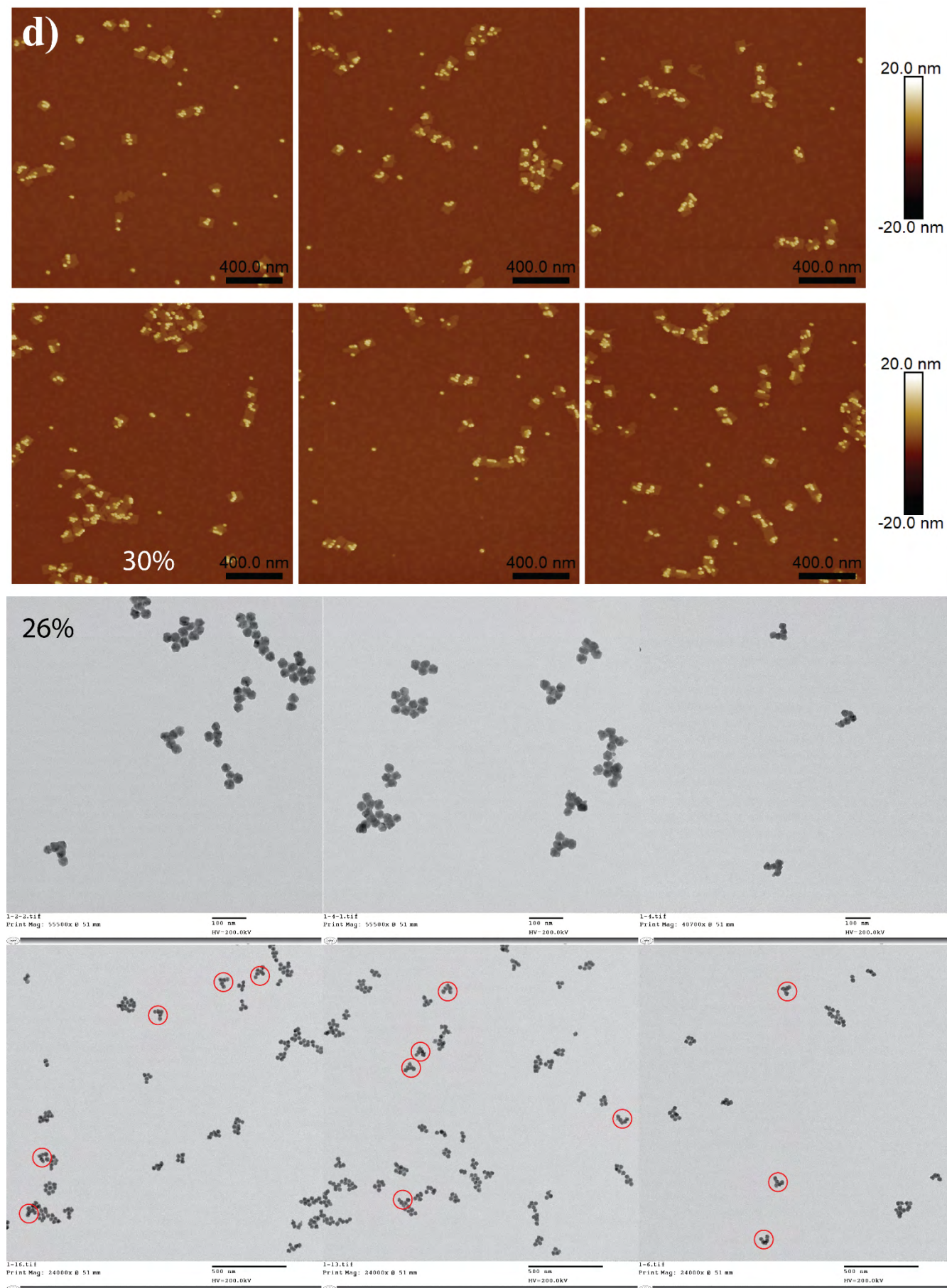




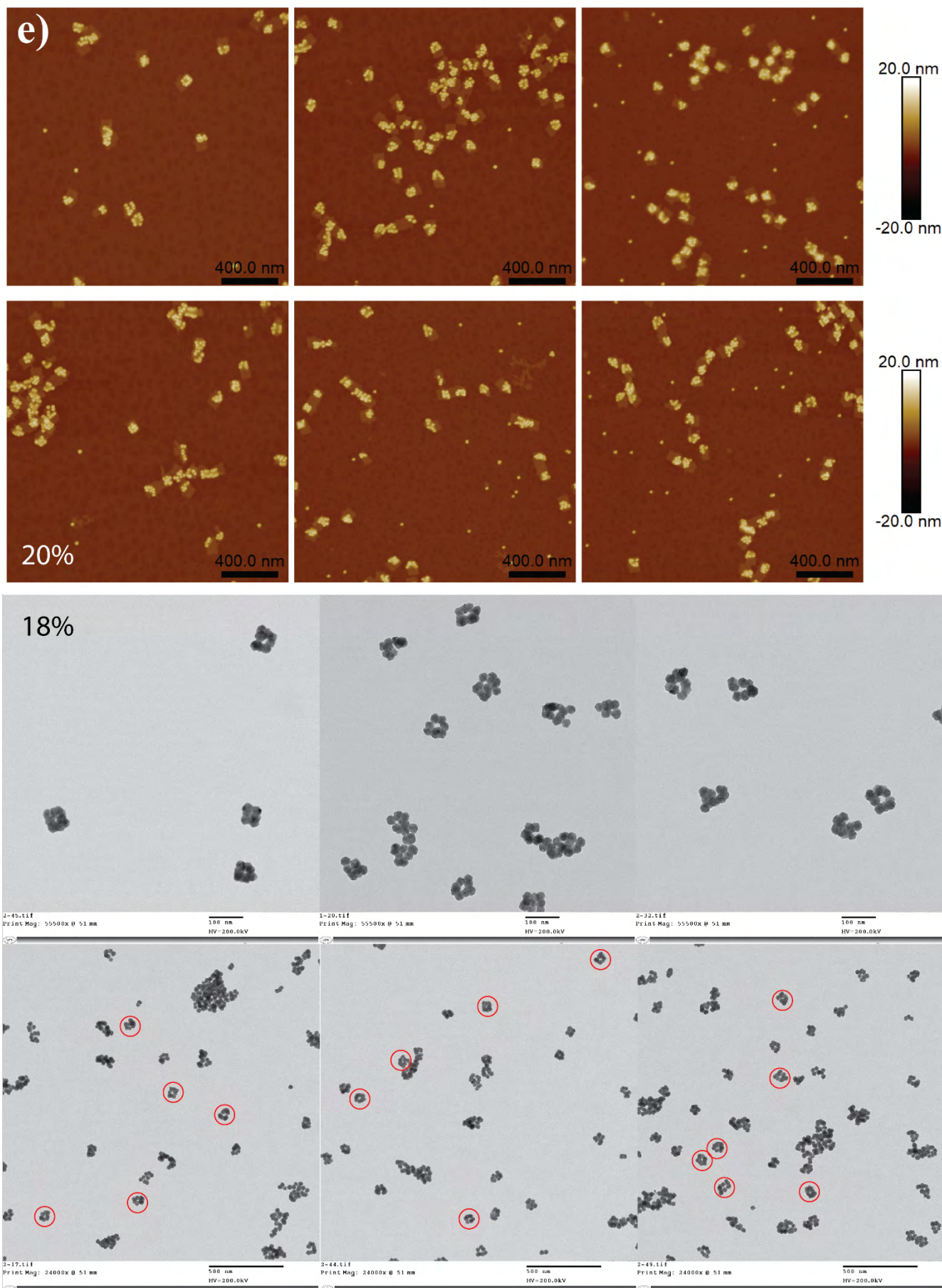


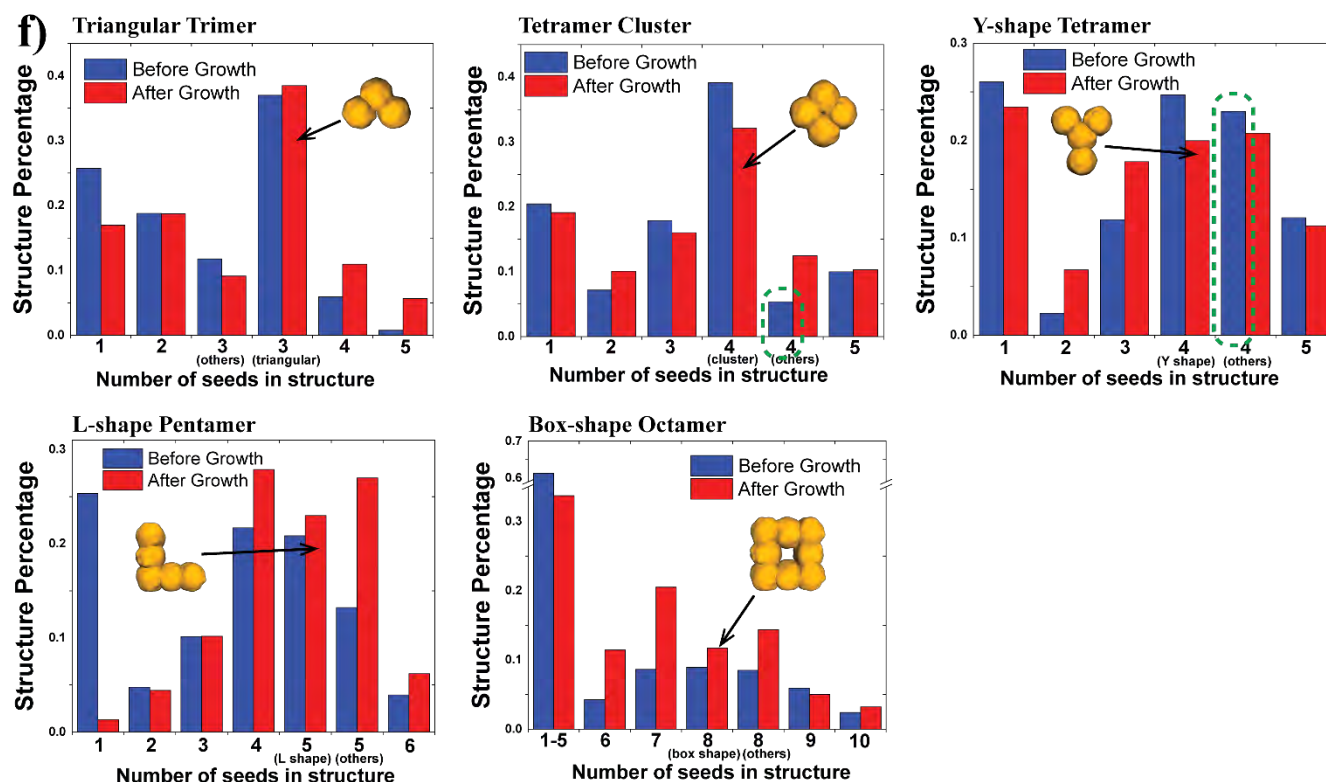




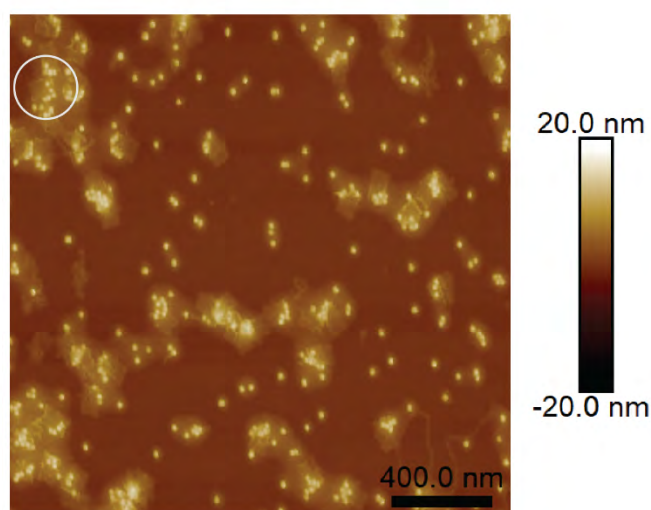
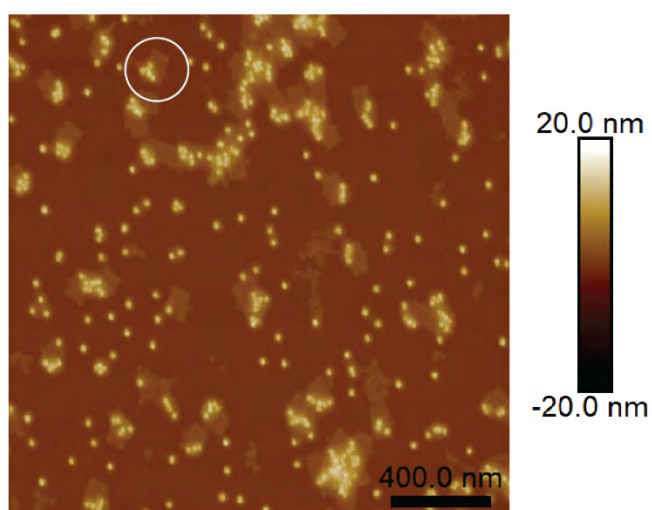
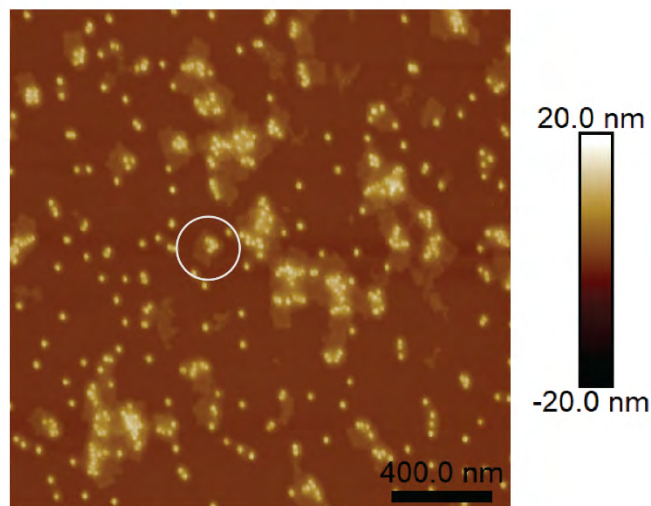
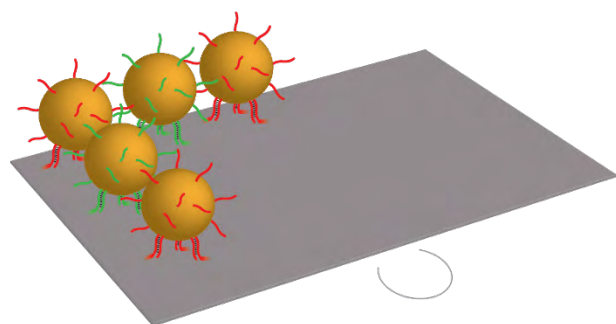








**Fig. S14.** Additional AuNP seed-origami assembly AFM images and AGLO crude product TEM images of the **a)** Triangular Trimer, **b)** Tetramer Cluster, **c)** Y-shape Tetramer, **d)** L-shape Pentamer and the **e)** Box-shape Octamer gold nanostructures. Yields counted from AFM images and TEM images are marked on the images respectively. **f)** The distribution of AuNP seed assemblies templated by the DNA origami before the gold growth (Blue, statistics from AFM images) and the distribution of AuNP structures in the crude product after the complete AGLO process (Red, statistics from TEM images). The TEM images of the L-shape and Box-shape structures are hard for the ImageJ algorithm to process; thus, their statistics are analyzed manually (N>300).



**Fig. S15.** The original L-shape Pentamer design where the AuNP seeds were positioned along the edge of the DNA origami template and its AFM images. Very few correctly assembled structures (white circle) can be observed from the AFM images.

## **S-X. AGLO strategy with Magnetic Beads**

### **1. MBs preparation**

Dynabeads™ MyOne™ with Carboxylic Acid functional groups (MB, cat. # 65011) was purchased from ThermoFisher Scientific and used as purchased. First, resuspend the MBs by rotating the vial for a few minutes and then transfer 100  $\mu\text{L}$  to an Eppendorf tube. Place the Eppendorf on a magnet for 2 min and remove the supernatant. Wash the MBs 2 times with 200  $\mu\text{L}$  MES buffer (0.1 M, pH 4.8) and resuspend in 100  $\mu\text{L}$  MES buffer. In a separate Eppendorf, mix 5 nmol LS strand (5' amine modified linker strand) and 40  $\mu\text{L}$  1.25 M EDC in MES buffer to a total volume of 100  $\mu\text{L}$ . Add the LS strand/EDC solution to the MBs, vortex for 10 seconds to mix and incubate on a rotator at room temperature for 3 hrs or overnight. After the incubation, wash the MBs 3 times with 200  $\mu\text{L}$  TT buffer (each time incubate for 30 min) and resuspend the MBs in 100  $\mu\text{L}$  Tris buffer. The functionalized MBs were stored at 4 °C.

MES buffer (pH 4.8) contains 0.1M 2-(N-morpholino)ethanesulfonic acid (MES) with a pH adjusted to 4.8 using NaOH. The Tris buffer contains 1 M tris(hydroxymethyl)aminomethane (Tris) with a pH adjusted to 8 using HCl. The TT buffer was prepared by mixing 12.5 mL of the Tris buffer (pH 8) with 50  $\mu\text{L}$  of 10% Tween-20 and bringing the volume to 50 ml using Milli-Q water.

### **2. Cycle I protocols**

a) Take 50  $\mu\text{L}$  functionalized MBs and wash 3 times with 200  $\mu\text{L}$  1 $\times$ HEPESMg buffer; remove the supernatant each time with the help of a magnet. b) After removing the supernatant for the last time, add 200  $\mu\text{L}$  AuNP seed-origami assembly solution containing 0.5 nM seed-origami assembly to disperse the MBs. Incubate the mixture on a rotator for 20 min on a rotator. c) Remove and collect the supernatant with the help of a magnet. The supernatant mainly contains the excess AuNP seeds and was reused for the next seed-origami assembly step. d) Wash the MBs with immobilized seed-origami 2 times with 1 $\times$ HEPESMg and 1 time with 1 $\times$ HEPESMg-PVP360 (contains PVP360 for gold growth). Resuspend the MBs in 200  $\mu\text{L}$  1 $\times$ HEPESMg-PVP360 buffer. e) Incubate the solution on a rotator in the cold room (4 °C) for 10 min, followed by the addition of 40  $\mu\text{L}$  gold source (for the Linear Trimer structure) and incubation in the cold room for 2 hrs. f) Move the solution to room temperature and incubate for an additional 15-30 min before adding 3  $\mu\text{L}$  OEG (0.1 M) and incubate for 10 min. g) Remove the supernatant after the incubation and wash the MBs one time with 1 $\times$ HEPESMg buffer. h) Remove the supernatant again and add 200  $\mu\text{L}$  8M Urea. Vortex and ultrasonicate a few times to lift-off the grown gold nanostructures from the MB surface. Separate and collect the AGLO product and the MBs respectively through magnetic separation. i) Wash the MBs a few times with water and buffer and the MBs can be used for the next round of the synthesis cycle.



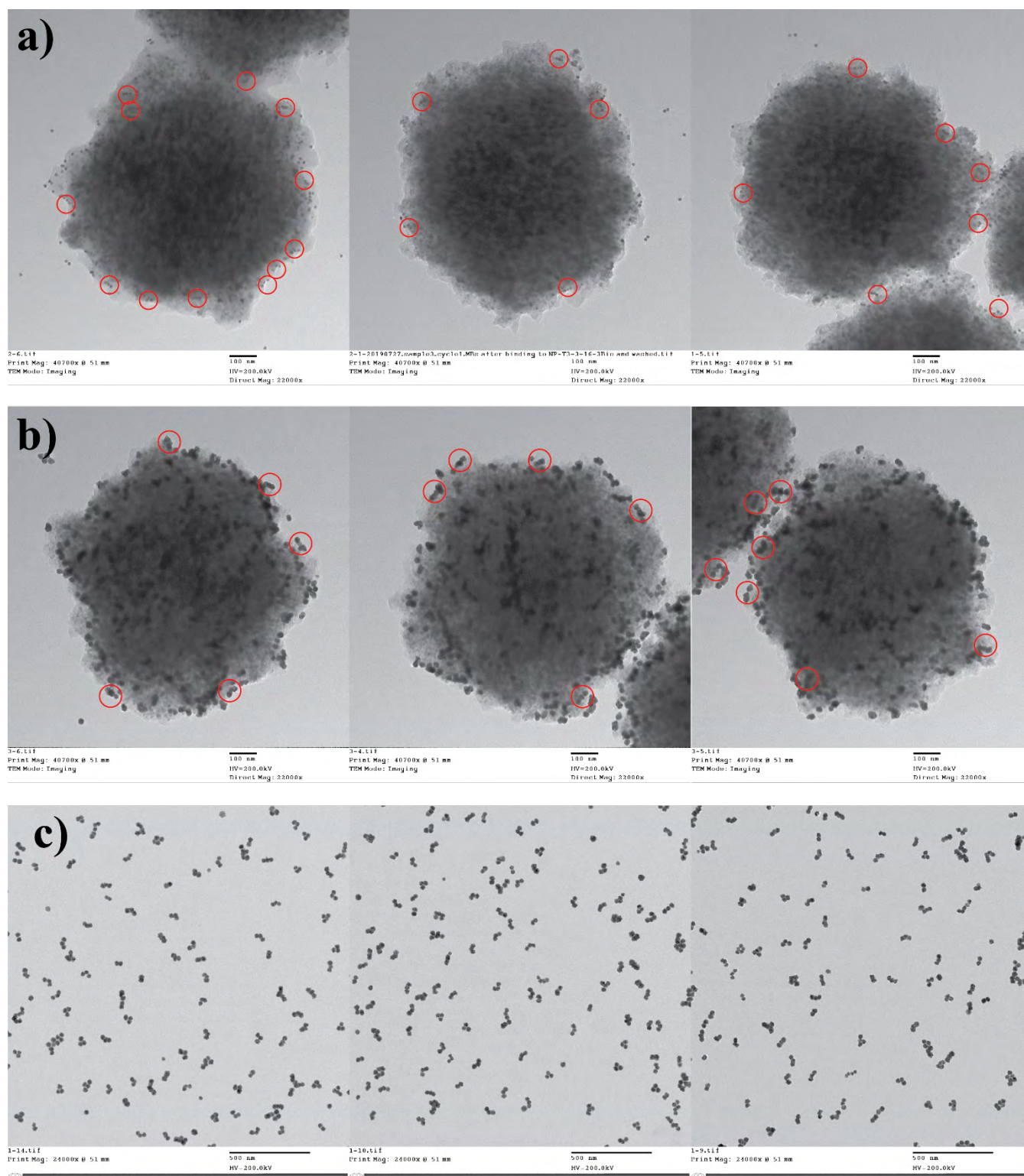
### 3. Cycle II protocols

Step a) to g) are the same as Cycle I.

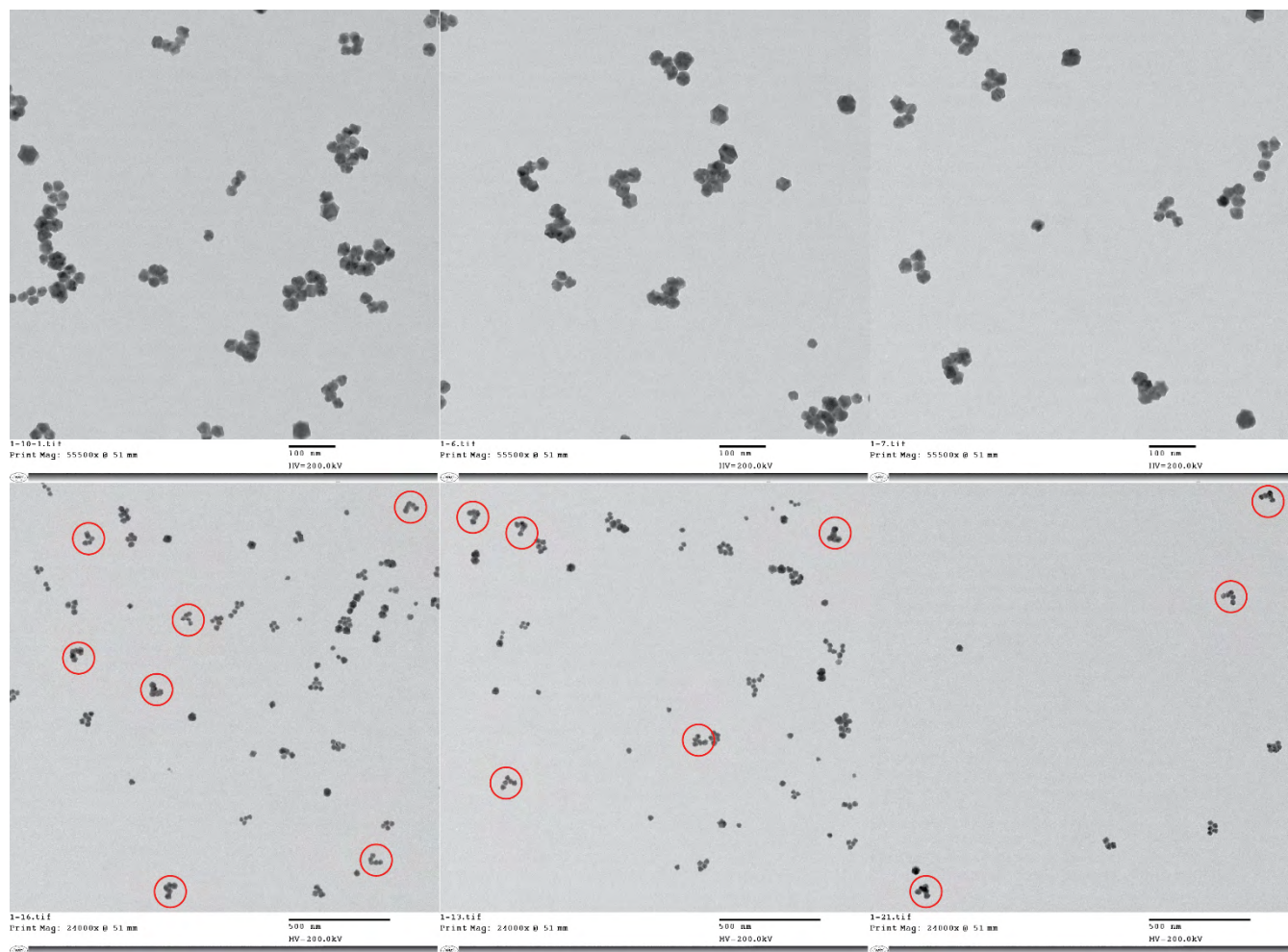
h) Remove the supernatant again and add 200  $\mu\text{L}$  invading strand solution containing 90  $\mu\text{L}$  BS1 comp (100  $\mu\text{M}$ ), 90  $\mu\text{L}$  BS2 comp (100  $\mu\text{M}$ ) and 20  $\mu\text{L}$  10 $\times$ HEPESMg buffer. i) Incubate the solution on a rotator for 2 days. j) Separate and collect the released AGLO product and the MBs respectively through magnetic separation. k) Wash the recovered MBs 3 times with 1 $\times$ HEPESMg buffer and resuspend the MBs in the AuNP seeds solution recovered from Step c) above. Incubate the solution on a rotator overnight for seed-origami assembly.

Repeat Step c) to Step h) as Cycle I.

Collect the 2-round AGLO product, wash and ultrasonicate 2 more times with Milli-Q water and characterize by TEM imaging. AuNPs with a diameter greater than 35 nm are evidently grown gold structures that had not been released in the first synthetic round and further grew to bigger sizes in the second gold growth step (Fig. S20b, green circle).

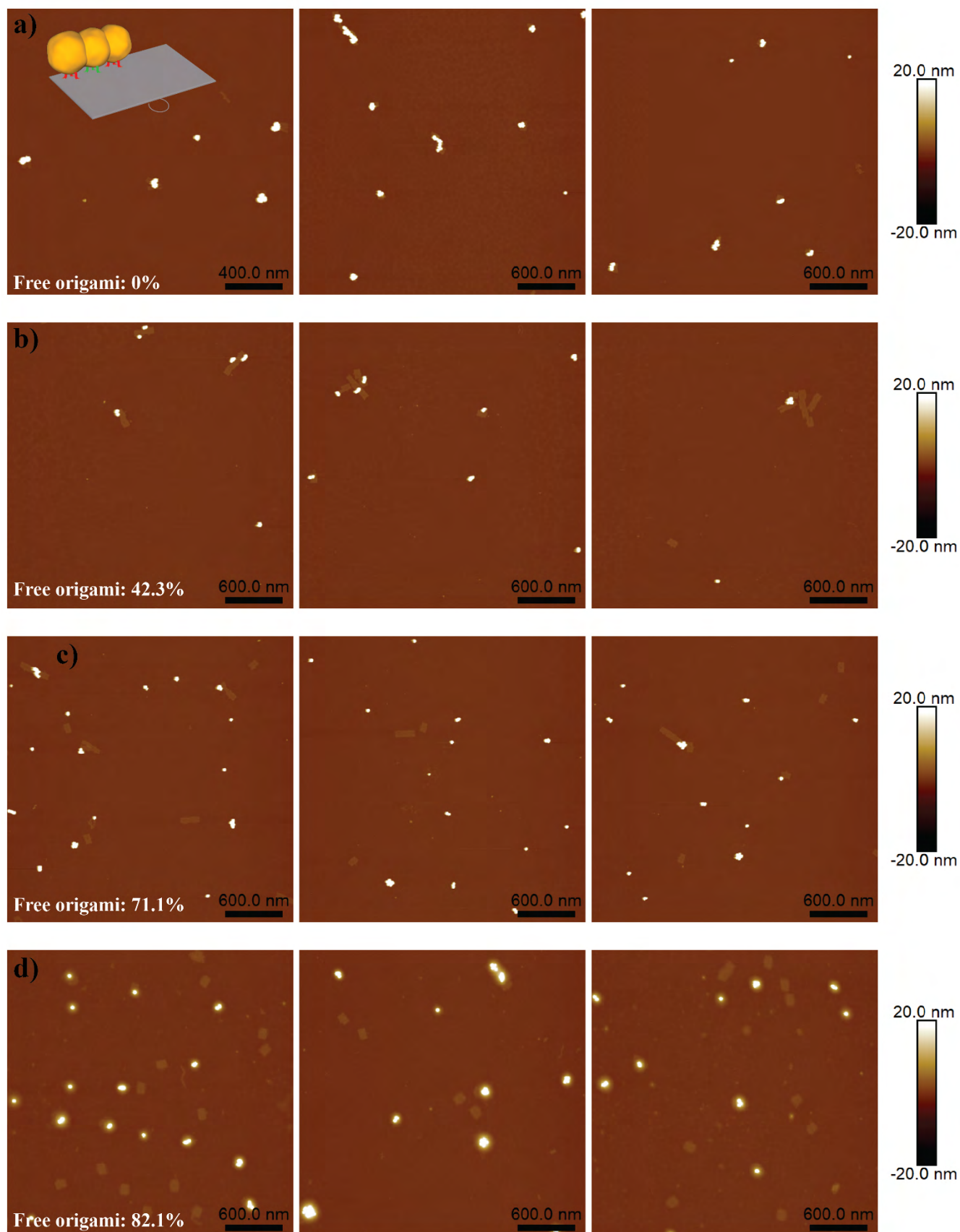


**Fig. S16. a)** Additional TEM images of the MBs after magnetic separation and buffer wash. The MBs were previously incubated with the Linear Trimer AuNP seed-origami assembly crude mixture with excess AuNP seeds. Linear Trimer structures that can be clearly distinguished are circled in red. **b)** Additional TEM images of the MBs after gold growth. Grown and merged Linear Trimer structures that can be clearly distinguished are circled in red. **c)** Additional TEM images of the Linear Trimer crude product Collected from the AGLO-MBs Cycle I process.



**Fig. S17.** TEM images of the L-shape Pentamer crude product collected from the AGLO-MBs Cycle I process.

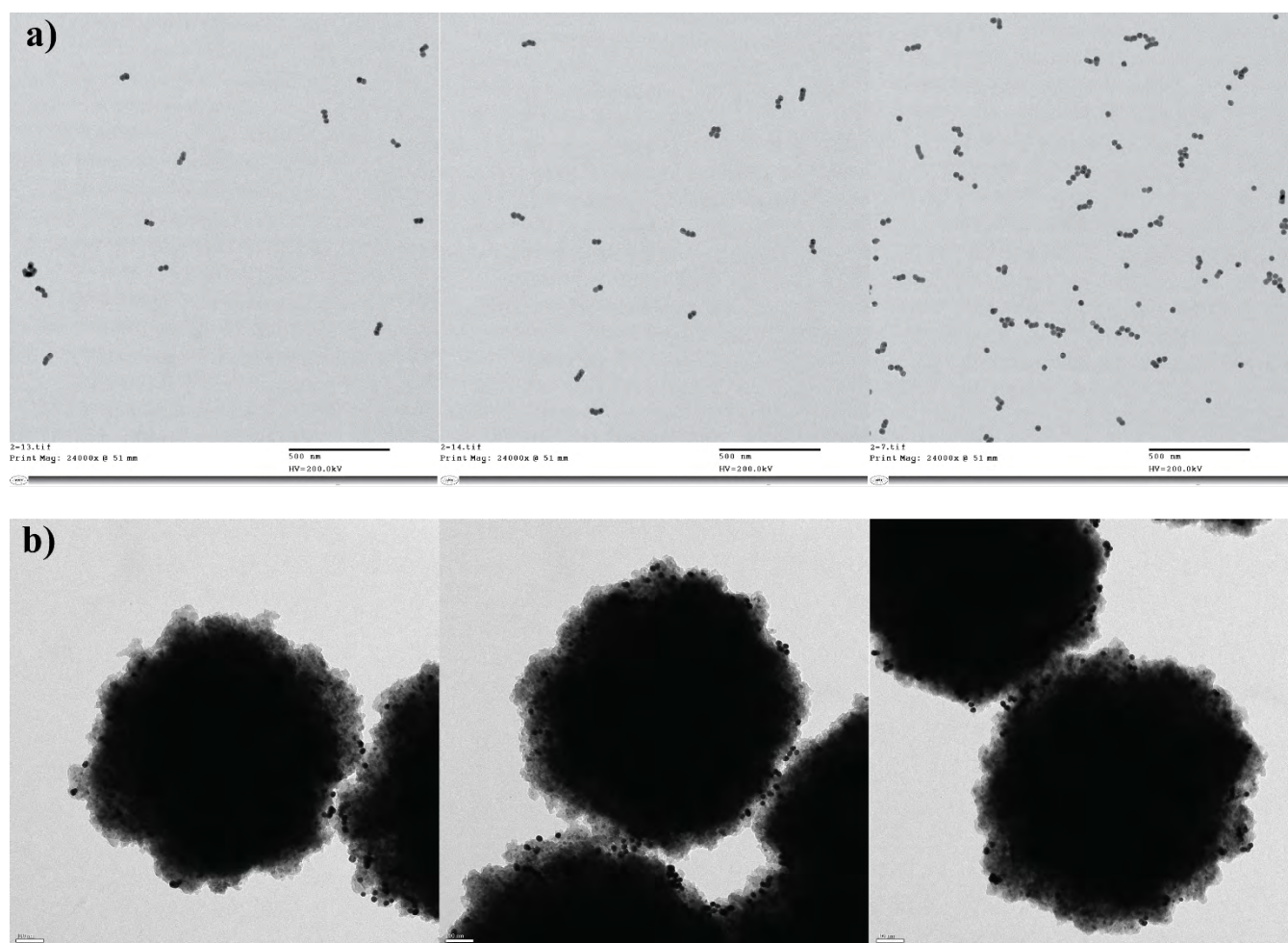




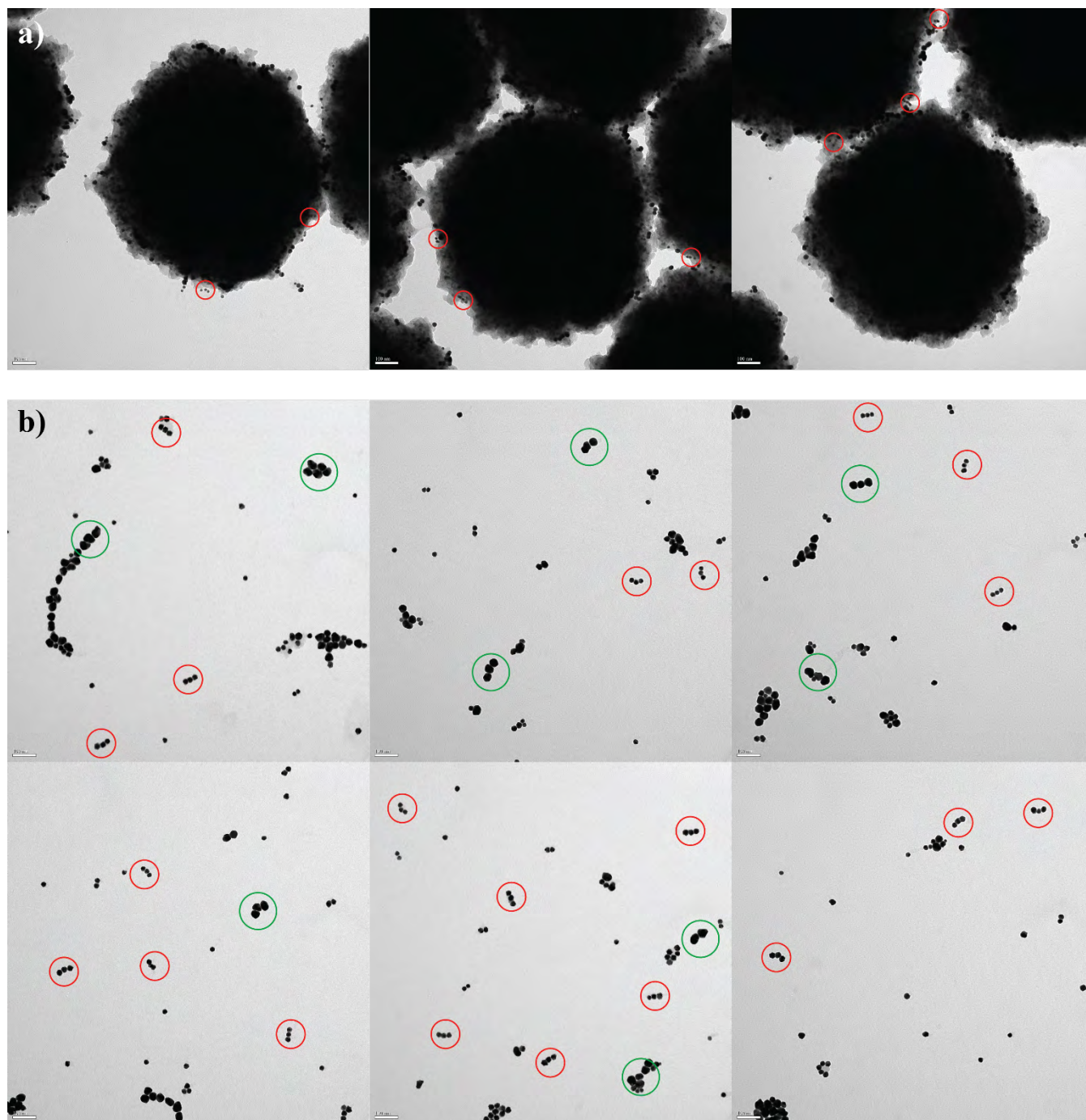
**Fig. S18.** AFM images of using strand displacement to lift off grown Linear Trimer gold nanostructures from the origami templates after the gold growth step. 500 times invading strands were applied. AFM images of the grown Linear Trimer gold nanostructure **a)** before strand displacement, **b)** after incubating



with the invading strands for 16 hrs at room temperature, **c)** after incubating with the invading strands for 64 hrs at room temperature and **d)** after incubating with the invading strands for 20 hrs at 37°C.



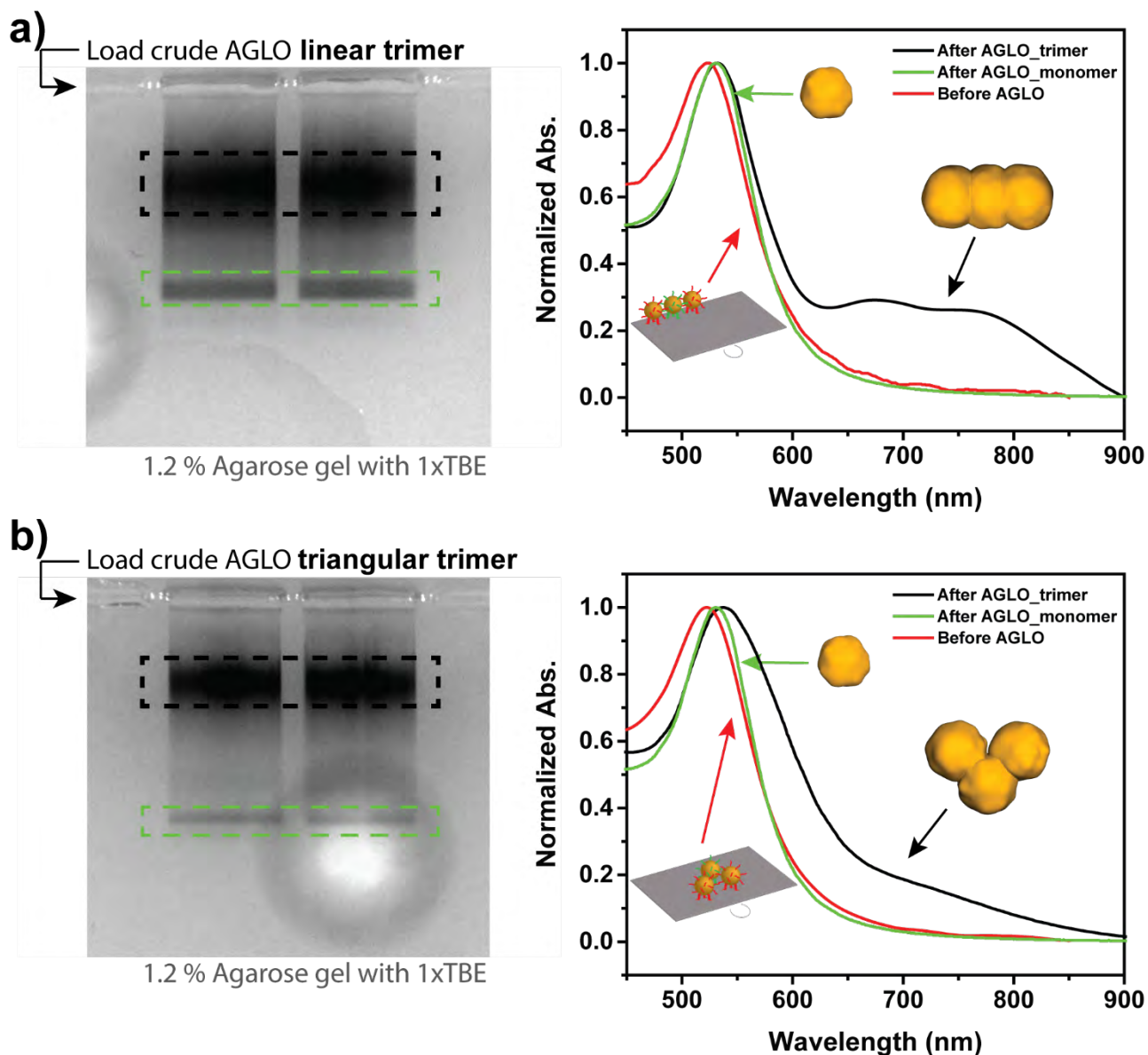
**Fig. S19.** The AGLO-MBs system, Cycle II, round 1. **a)** TEM images of the AGLO crude product lifted off through strand displacement. **b)** TEM images of the MBs after strand displacement and buffer wash. Grown gold nanostructures were not fully released from the MBs.



**Fig. S20.** The AGLO-MBs system, Cycle II, round 2. **a)** Re-using the MBs after strand displacement releasing of the grown gold nanostructures and regenerating the surface bound DNA origami template. TEM images show the MBs (with the regenerated DNA origami templates) after incubation with the recycled AuNP seeds. AuNP seeds that assembled in a Linear Trimer structure that can be clearly distinguished are circled in red. **b)** The crude product from AGLO-MBs Cycle II round 2. Grown Linear Trimer structures from the second synthetic round are circled in red and grown Linear Trimer structures from the first synthetic round but failed to release (which continued to grow bigger in the second round) are circled in green.

## S-XI. Additional results and discussions

### 1. UV-vis spectra of the linear trimer and the triangular trimer.

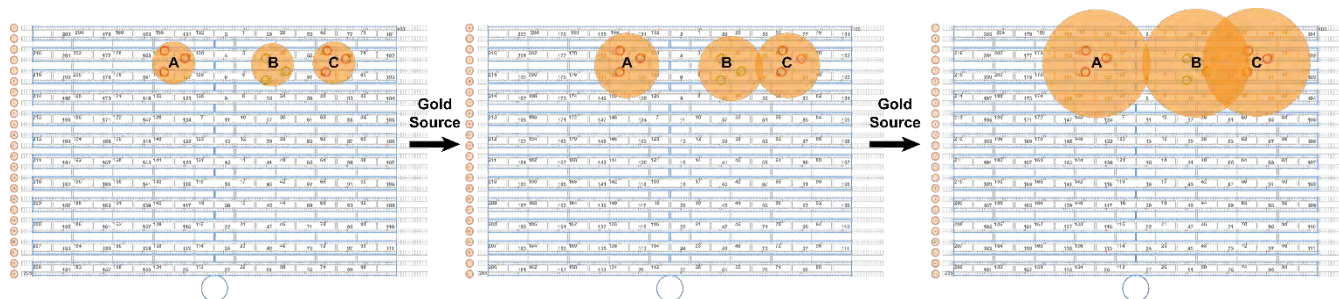


**Fig. S21.** The gel purification and UV-vis spectra of **a)** the linear trimer and **b)** the triangular trimer structure before and after the AGLO process. Crude product of the two structures were loaded on a 1.2% agarose gel with 1xTBE buffer and run for 40 min under 100V. After gel electrophoresis, the gel bands corresponding to the grown monomers (green boxes) and trimers (black boxes) were excised and the product eluted from the gel for UV-vis measurement. Comparing to seeded structures before growth (red curves), the plasmonic peak of AuNP monomers after gold growth shifted slightly to longer wavelength ( $\sim 530$  nm), which is consistent to the size dependence of the spherical AuNP absorptions. As for merged trimer structures (black curves), longitudinal plasmonic resonances can be observed in longer wavelength region (wavelength  $> 600$  nm), as conductive junctions form between AuNP seeds and the aspect ratio of the gold structure changes.



## 2. The influence of AuNP seed distance on the merging of gold nanostructures.

The influence of the AuNP seed distance on the merging of the gold nanoparticle is intricate as the seed distance is not an independent factor that affects the merging. Several other co-factors will also take effect when the seed distance is changed.

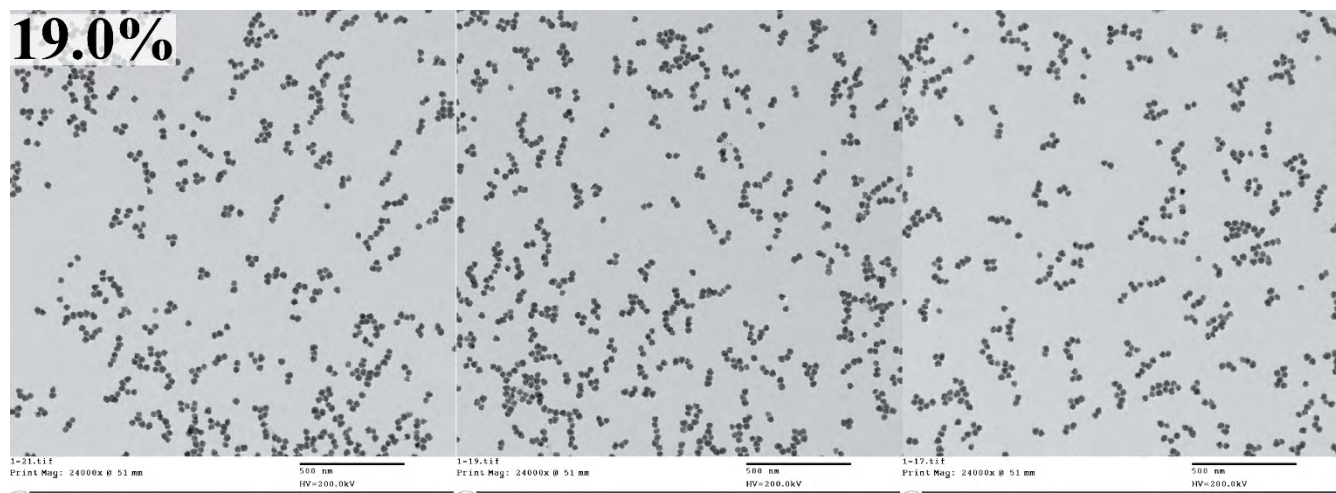


**Fig. S22.** A schematic illustration of the influence of gold source and seed distance on the merging in the AGLO growth process.

1) Amount of gold source applied (i.e. the final particle size). As shown in **Fig. S22**, when a limited amount of gold source is added to grow the AuNP seed, only the seed sitting close to each other (B and C) have the chance of merging as their growing surfaces start to contact each other as their size increases. However, if more gold source is added, theoretically at one point the size of the growing AuNP seeds will allow the contact of the two seeds with a longer distance (A and B) and subsequently enable their merging. In summary, whether or not the seeds will merge when the distance between seeds increases depends on how big the seeds will be grown. 2) Seed distance comparing to the PVP360 molecule size. Based on our results of the influence of the surfactant molecule to AuNP merging, if the seed distance is big enough for the molecule of PVP360 to diffuse in between the seeds, the merging of the AuNPs will be hindered even if the seeds were grown big enough for the NP to be in contact. 3) Template deformation. The single layer DNA origami template will experience more and more strain and deformation when AuNP seeds are grown bigger and bigger, which will affect the merging yield of the structure, as discussed in supporting information S-IX.

### 3. AGLO with 1:1 mixture of PVP360 and PVP10 as surfactant.

An experiment of the AGLO synthesis of the linear trimer structure was carried out with a 1:1 (mass) mixture of PVP360 and PVP10 as surfactants, instead of only with PVP360 as the surfactant in a standard procedure.



**Fig. S23.** TEM images of the crude product from the AGLO synthesis of the linear trimer structure with a 1:1 (mass) mixture of PVP360 and PVP10 as surfactants. The merging yield (19.0%) of the linear trimer is still significantly lower than that of the experiment with only PVP360 (47%) as a surfactant. This is in consistence with our assumption that PVP10 will diffuse into the gaps of the AuNP seeds and hinder the merging of growing NPs, even when PVP360 is present.

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## Unmodified staple strands (1-216)

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3 TGCCTTGACTGCCTATTTCCGAACAGGGATAG  
4 GAGCCGCCCCACCACCGGAACCGCGACGGAAA  
5 AACCAGAGACCCTCAGAACCGCCAGGGGTCAG  
6 TTATTCATAGGGAAGGTAAATATTCATTCAGT  
7 CATAACCCGAGGCATAGTAAGAGCTTTTTAAG  
8 ATTGAGGGTAAAGGTGAATTATCAATACCGG  
9 AAAAGTAATATCTTACCGAAGCCCTTCCAGAG  
10 GCAATAGCGCAGATAGCCGAACAATTCAACCG  
11 CCTAATTTACGCTAACGAGCGTCTAATCAATA  
12 TCTTACCAGCCAGTTACAAAATAAATGAAATA  
13 ATCGGCTCGGAGCATGTAGAAACCTATCATAT  
14 CTAATTTATCTTTCCTTATCATTCATCTGAA  
15 GCGTTATAGAAAAAGCCTGTTTAGAAGGCCGG  
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110	GATGGCAATTTTAATCAATATCTGGTCACAAAATATC	153	ATACGTAAAAGTACAACGGAGATTTTCAATCAAG
111	AAACCCCTCTTTACCAGTAATAAAAGGGATTCCACAGTCACACGTTTT	154	CAATGACACTCCAAAAGGAGCCTTACAACGCC
112	CCGAAATCCGAAAATCCTGTTTGAAGCCGGAA	155	AAAAAAGGACAACCATCGCCCACGCGGGTAAA
113	CCAGCAGGGGCAAAATCCCTTATAAAGCCGGC	156	TGTAGCATTCCACAGACAGCCCTCATCTCCAA
114	GCATAAAGTCCACACAACATACGAAGCGCCA	157	GTAAAGCACTAAATCGGAACCTAGTTGTTC
115	GCTCACAATGTAAAGCCTGGGGTGGGTTTGCC	158	AGTTTGAGGCCCTTACCGCTGGTTGCGCTC
116	TTCGCCATTGCCGGAACACAGGCATTAATCA	159	AGCTGATTACAAGAGTCCACTATTGAGGTGCC
117	GCTTCTGGTCAGGCTGCGCAACTGTGTTATCC	160	ACTGCCCGCCGAGCTCGAATTCGTTATTACGC
118	GTTAAATTTTAACCAATAGGAACCCGGCACC	161	CCCGGGTACTTTCCAGTCGGGAAACGGGCAAC
119	AGACAGTCATTCAAAAGGGTGAGAAGCTATAT	162	CAGCTGGCGGACGACGACAGTATCGTAGCCAG
120	AGGTAAAGAAATCACCATCAATATAATATTTT	163	GTTTGAGGGAAAGGGGGATGTGCTAGAGGATC
121	TTTCATTTGGTCAATAACCTGTTTATATCGCG	164	CTTTCATCCCCAAAAACAGGAAGACCGGAGAG
122	TCGCAATGGGGCGCGAGCTGAAATAATGTGT	165	AGAAAAGCAACATTAATGTGAGCATCTGCCA
123	TTTTAATIGCCGAAAGACTTCAAAACACTAT	166	GGTAGCTAGGATAAAAAATTTTGTGTTAATC
124	AAGAGGAACGAGCTTCAAAGCGAAGATACATT	167	CAACGCAATTTTIGAGAGATCTACTGATAATC
125	GGAATTACTCGTTTACCAGACGACAAAAGATT	168	CAATAAATACAGTTGATTCCCAATTTAGAGAG
126	GAATAAGGACGTAAACAAAGTGCTCTAAAACA	169	TCCATATACATACAGGCAAGGCAACTTTATTT
127	CCAAATCACTTGCCCTGACGAGAACGCCAAAA	170	TACCTTTAAGGTCCTTACCCTGACAAAGAAGT
128	CTCATCTTGAGGCAAAAGAATACAGTGAATTT	171	CAAAAATCATTGCTCCTTTTGATAAGTTTICAT

172	TTTGCCAGATCAGTTGAGATTAGTGGTTTAA	215	ACGGTCAATTTTGACAGCATCGGAACGAACCTCAG
173	AAAGATTCAGGGGGTAATAGTAAACCATAAAT	216	CAGCGAAATTTAACTTTCAACAGTTTCTGGGATTTTGCTAAACTTTT
174	TTTCAACTATAGGCTGGCTGACCTTGTATCAT		
175	CCAGGCGCTTAATCATTGTGAATTACAGGTAG		
176	CGCCTGATGGAAGTTTCCATTAAACATAACCG		
177	TTTCATGAAAAATTGTGTCGAAATCTGTACAGA		
178	ATATATTCTTTTTTCACGTTGAAAAATAGTTAG		
179	AATAATAAGGTCGCTGAGGCTTGCAAAGACTT		
180	CGTAACGATCTAAAGTTTGTGCTGAATTGCG		
181	ACCCAAATCAAGTTTTTTGGGGTCAAAGAACG		
182	TGGACTCCCTTTTCACCAGTGAGACCTGTCTGT		
183	TGGTTTTTAACGTCAAAGGGCGAAGAACCATC		
184	GCCAGCTGCCTGCAGGTCGACTCTGCAAGGCG		
185	CTTGCATGCATTAATGAATCGGCCGCCAGGG		
186	ATTAAGTTCGCATCGTAACCGTGCAGTAACA		
187	TAGATGGGGGGTAACGCCAGGGTTGTGCCAAG		
188	ACCCGTCGTCATATGTACCCCGGTAAAGGCTA		
189	CATGTCAAGATTCTCCGTGGGAACCGTTGGTG		
190	TCAGGTCACTTTTCGGGAGAAGCAGAATTAG		
191	CTGTAATATTGCCTGAGAGTCTGGAAAAC TAG		
192	CAAAATTAAAGTACGGTGTCTGGAAGAGGTCA		
193	TGCAACTAAGCAATAAAGCCTCAGTTATGACC		
194	TTTTTGCGCAGAAAACGAGAATGAATGTTTAG		
195	AAACAGTTGATGGCTTAGAGCTTATTTAAATA		
196	ACTGGATAACGGAACAACATTATTACCTTATG		
197	ACGAACTAGCGTCCAATACTGCGGAATGCTTT		
198	CGATTTTAGAGGACAGATGAACGGCGCGACCT		
199	CTTTGAAAAGAACTGGCTCATTATTTAATAAA		
200	GCTCCATGAGAGGCTTTGAGGACTAGGGAGTT		
201	ACGGCTACTTACTTAGCCGGAACGCTGACCAA		
202	AAAGGCCGAAAGGAACAATAAGCTTTCCAG		
203	GAGAAATAGCTTTTGCGGGATCGTCGGGTAGCA		
204	ACGTTAGTAAATGAATTTTCTGTAAGCGGAGT		
205	TTTTCGATGGCCCACTACGTAAACCGTC		
206	TATCAGGGTTTTTCGGTTTTCGTATTGGAACGCGCG		
207	GGGAGAGGTTTTTGTAAAACGACGGCCATTCCAGT		
208	CACGACGTTTTTGTAAATGGGATAGGTCAAAACGGCG		
209	GATTGACCTTTTGATGAACGGTAATCGTAGCAACA		
210	AGAGAATCTTTGGTTGTACCAAAAACAAGCATAAA		
211	GCTAAATCTTTCTGTAGCTCAACATGTATTGCTGA		
212	ATATAATGTTTTCATTGAATCCCCCTCAAATCGTCA		
213	TAAATATTTTTTGAAGAAAAATCTACGACCAGTCA		
214	GGACGTTGTTTTTCATAAGGGAACCGAAAGGCGCAG		