

Supplementary Information

DNA origami directed 3D nanoparticle superlattices via electrostatic assembly

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1. Materials

All reagents were commercially available and applied without any further purification. In all procedures, the water used was Milli-Q purified. 50× TAE Buffer (2 M tris(hydroxymethyl)aminomethane (Tris), 50 mM ethylenediaminetetraacetic acid (EDTA) and 1 M acetic acid for adjusting the pH to 8.0) was purchased from VWR Chemicals. Ethidium bromide was purchased from Sigma-Aldrich, agarose from Bioline and 6× Gel Loading Dye Blue from New England Biolabs. The single-stranded scaffolds derived from bacteriophage M13mp18 (7249 nt and 7560 nt) were purchased from Tilibit Nanosystems, whereas the short single-stranded staple strands used in the DNA origami folding were purchased from Integrated DNA Technologies.

2. DNA origami

Three different DNA origami nanostructures (6HB, 24HB and 60HB) were prepared. The fabrication process varied slightly between the structures, but for all structures, the same purification steps and analysis techniques were applied.

2.1. 6-helix bundle (6HB)

The 6-helix bundle (6HB) design is adapted from Bui *et al.*^{S1}, and the original set of the 170 oligonucleotide staple strands used for the folding are listed in the same reference. In the design used in this study, the extended staple strands, originally used for quantum dot attachment, have been replaced with shorter ones leaving no overhangs to the 6HB.

The 6HB was folded in 100 µL quantities in a one-pot reaction by mixing a 7249 nucleotides long single-stranded scaffold derived from bacteriophage M13mp18 (final concentration of 20 nM in the folding mixture) with ten times excess of staple strands. The folding took place in a buffer containing 1× TAE (40 mM tris(hydroxymethyl)aminomethane (Tris), 1 mM ethylenediaminetetraacetic acid (EDTA), and acetic acid for adjusting the pH to 8.0) and 12.5 mM MgCl₂. The DNA origami folding mixture was subjected to a thermal-annealing ramp (G-storm G1 Thermal Cycler) that first cooled from 90 °C to 70 °C over the course of 13.2 minutes, from 70 °C to 60 °C over 13.2 minutes, and then slowly cooled from 60 °C to 27 °C over 10 hours and 52 minutes. After the annealing, the DNA origami structures were stored at 12 °C until the program was manually stopped. After the folding procedure, the DNA origami structures were refrigerated at 4 °C.

2.2. 24-helix bundle (24HB)

The 24-helix bundle (24HB) design and folding conditions are with some small adjustments adapted from Kuzyk *et al.*^{S2}, and all of the 175 oligonucleotide staple strands used for the folding of the structure are listed in the same reference (*left-handed 24HB design* in ^{S2}).

The 24HB was folded in 100 μ L quantities in a one-pot reaction by mixing a 7560 nucleotides long single-stranded scaffold derived from bacteriophage M13mp18 (final concentration of 10 nM in the folding mixture) with ten times excess of staple strands. The folding took place in a buffer containing 1 $\times$ TAE (40 mM tris(hydroxymethyl)aminomethane (Tris), 1 mM ethylenediaminetetraacetic acid (EDTA), and acetic acid for adjusting the pH to 8.0) and 14 mM MgCl₂. The DNA origami folding mixture was subjected to a thermal-annealing ramp (G-storm G1 Thermal Cycler) that first cooled from 65 °C to 60 °C over the course of 90 minutes, and then slowly cooled from 60 °C to 39 °C over 60 hours. After the annealing, the DNA origami structures were stored at 12 °C until the program was manually stopped. After the folding procedure, the DNA origami structures were refrigerated at 4 °C.

2.3. 60-helix bundle (60HB)

The 60-helix bundle (60HB) design and folding conditions are adapted from Linko *et al.*⁵³, and all of the 141 oligonucleotide staple strands used for the folding of the structure are listed in the same reference.

The 60HB was folded in 100 μ L quantities in a one-pot reaction by mixing a 7249 nucleotides long single-stranded scaffold derived from bacteriophage M13mp18 (final concentration of 20 nM in the folding mixture) with ten times excess of staple strands. The folding took place in a buffer containing 1 $\times$ TAE (40 mM tris(hydroxymethyl)aminomethane (Tris), 1 mM ethylenediaminetetraacetic acid (EDTA), and acetic acid for adjusting the pH to 8.0), 20 mM MgCl₂ and 5 mM NaCl. The DNA origami folding mixture was subjected to a thermal-annealing ramp (G-storm G1 Thermal Cycler) that first cooled from 65 °C to 60 °C over the course of 90 minutes, and then slowly cooled from 60 °C to 39 °C over 60 hours. After the annealing, the DNA origami structures were stored at 12 °C until the program was manually stopped. After the folding procedure, the DNA origami structures were refrigerated at 4 °C.

2.4. Purification of DNA origami

A non-destructive PEG purification method was used to remove the excess amount of staple strands from all the synthesized DNA origami structures (see also Fig. S1a, Fig. S2a and Fig. S3a). The PEG purification was carried out as reported by Stahl *et al.*⁵⁴, and the purifications steps are described in detail below:

- 400 μ L of the DNA origami reaction mixture was diluted four-fold in 1 $\times$ folding buffer (FOB) to obtain a starting volume of 800 μ L.
- The solution was mixed 1:1 with precipitation buffer (15 % PEG 8000 (w/v), 1 $\times$ TAE and 505 mM NaCl) and mixed thoroughly.
- The mixture was centrifuged at 14 000 rcf for 30 minutes at room temperature using an Eppendorf 5424R microcentrifuge.
- The supernatant was discarded and the DNA origami precipitate resuspended in 1 $\times$ FOB. Depending on the desired DNA origami concentration, the DNA origami precipitate was resuspended to 0.12-1 times the initial reaction volume.
- The precipitate was let to dissolve overnight at room temperature before use/refrigerated at 4 °C.

2.5. DNA origami concentration

The DNA origami concentration (c_{DNA}) was estimated using UV/Vis spectroscopy and Beer-Lambert law

$$A_{260} = \varepsilon_{260} c_{DNA} l,$$

where A_{260} is the absorbance at wavelength of 260 nm, ε_{260} is the approximated molar extinction coefficient and l is the path length through the solution in centimeters (0.05 cm). The extinction coefficient is approximated by the number of hybridized respective non-hybridized nucleotides in the DNA origami structure,⁵⁵ and the extinction coefficient was estimated to $0.8 \times 10^8 \text{ M}^{-1}\text{cm}^{-1}$ for the DNA origami 24HB shape, $0.9 \times 10^8 \text{ M}^{-1}\text{cm}^{-1}$ for the DNA origami 60HB shape and $1.0 \times 10^8 \text{ M}^{-1}\text{cm}^{-1}$ for the DNA origami 6HB shape. The absorbance at a wavelength of 260 nm was measured with a BioTek Eon Microplate spectrophotometer or a BioTek Cytation 3 Microplate reader using a Take3™ micro-volume plate and a sample size of 2 μL .

2.6. Agarose gel electrophoresis

Agarose gel electrophoresis was used to qualitatively analyze the DNA origami folding and confirm the removal of excess amount of staple strands. For the agarose gel electrophoresis, a 2 % (w/v) agarose gel was made by dissolving 2 g of agarose into 100 mL 1× TAE buffer containing 11 mM MgCl_2 . The gel was stained with 80 μL of ethidium bromide (EtBr) solution (0.58 mg mL^{-1}). The samples were prepared by mixing 10 μL of DNA origami solution with 2 μL of Gel Loading Dye Blue (6×), and the whole sample of 12 μL was loaded into the gel well. The M13mp18 scaffold strand was used as a reference sample after first diluted 2:3 in 1× FOB. The gel electrophoresis was performed at a constant voltage of 95 V for 40-60 minutes using a BioRad PowerPac™ Basic. 1× TAE buffer containing 11 mM MgCl_2 was used as a running buffer. The gel was kept in an ice bath for the run and it was visualized under UV light using a BioRad Gel Doc™ Ez Imager.

2.7. Transmission electron microscopy (TEM)

The folded and PEG-purified DNA origami nanostructures were imaged using transmission electron microscopy (TEM) to verify that they were correctly folded. The TEM images were obtained using a FEI Tecnai 12 Bio-Twin instrument operated at an acceleration voltage of 120 kV. The samples were prepared on plasma cleaned (30 seconds oxygen plasma flash) Formvar carbon coated copper grids (Electron Microscopy Science) mainly following the protocol described by Castro *et al.*⁵⁶. 3 μL of DNA origami solution ($c = 9\text{-}11 \text{ mM}$) was applied onto the carbon-coated side of the TEM grid and excess sample solution was blotted away with filter paper after an incubation of 2 minutes. The samples were further negatively stained using a 2 % uranyl formate stain solution containing 25 mM NaOH. First, the sample-side of the grid was immersed into a 5 μL stain solution droplet. The stain was blotted away with filter paper immediately, and the sample-side of the grid was immersed into another droplet of 20 μL of stain solution. Excess stain solution was blotted away with filter paper after 45 seconds. After these procedures, the sample were left to dry under ambient conditions for at least one hour before imaging.

2.8. Characterization of DNA origami structures

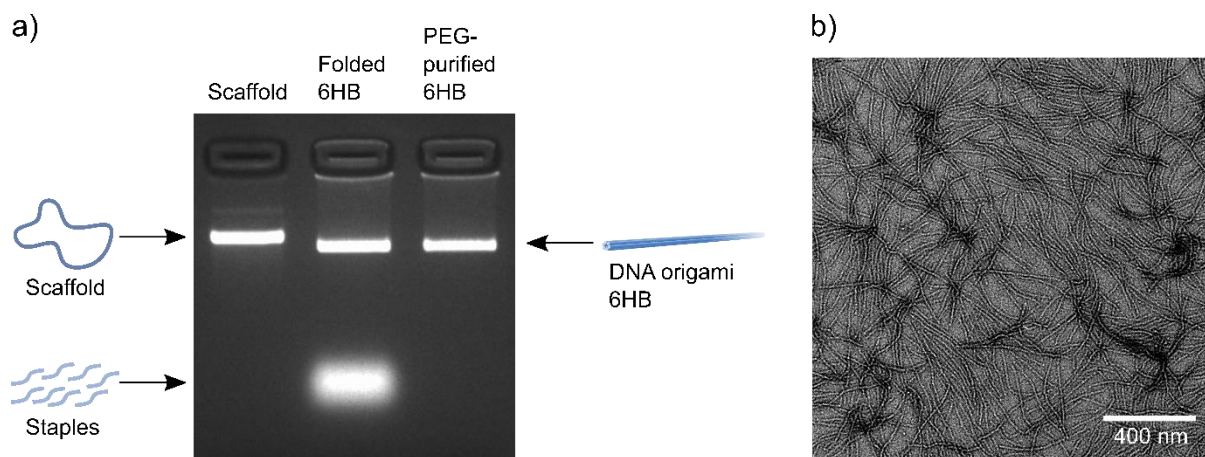


Fig. S1. a) Agarose gel electrophoresis of the folded 6HB structure before and after PEG-purification. The 7249 nt long single-stranded scaffold was used as reference. b) TEM image of the 6HB (negatively stained with uranyl formate).

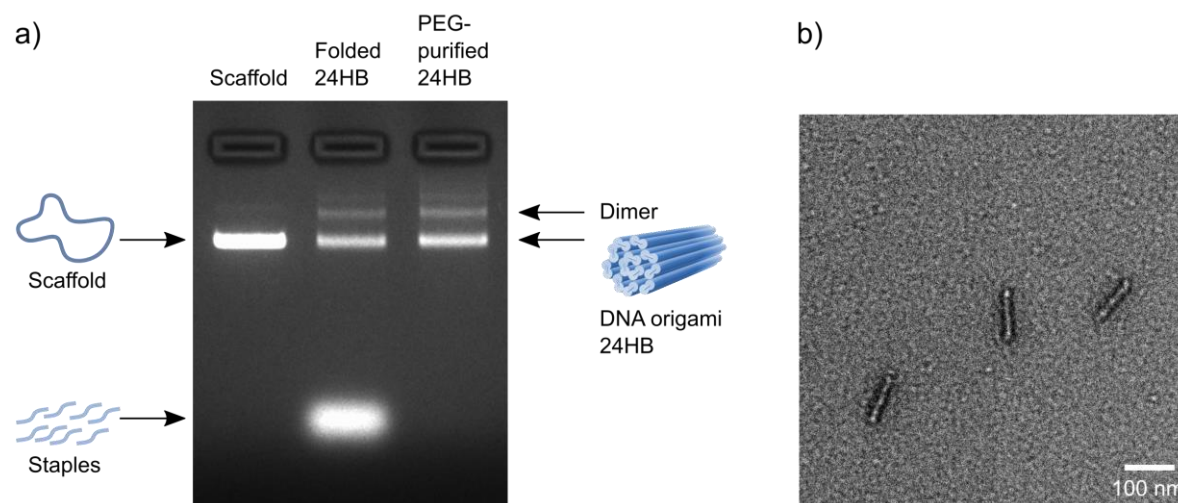


Fig. S2. a) Agarose gel electrophoresis of the folded 24HB before and after PEG-purification. The 7560 nt long single-stranded scaffold was used as reference. b) TEM image of the 24HB (negatively stained with uranyl formate).

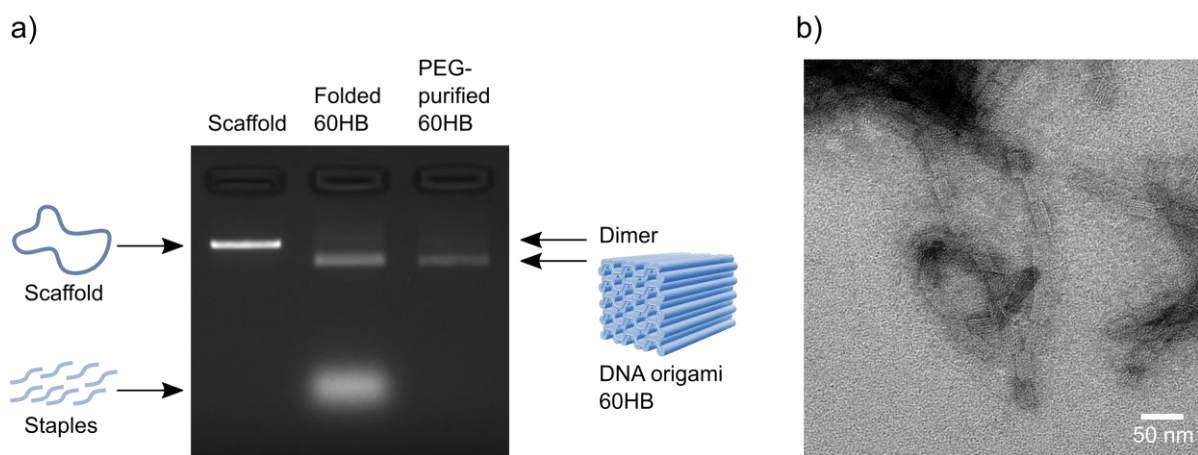


Fig. S3. Agarose gel electrophoresis of the folded 60HB before and after PEG-purification. The 7249 nt long single-stranded scaffold was used as reference. b) TEM image of the 60 HB (negatively stained with uranyl formate).

The 24HB and the 60HB structures have remarkably larger helical cross-section than the 6HB structure, and are therefore prone to form dimers and trimers during folding and purification. This can be observed as faint additional bands in the gel images. However, these oligomers are formed through blunt-end stacking at the helical interfaces of the structures and will therefore not prevent the AuNPs to bind to the DNA origami structures.

3. Cationic gold nanoparticles (AuNPs)

Cationic gold nanoparticles (AuNPs) of three different sizes were synthesized, all with a narrow size distribution. The larger two AuNPs, with an average core diameter, D_{core} , of 10.9 nm respective 12.4 nm (characterized with TEM), were synthesized as previously described by Hassinen *et al.*⁵⁷ and Liljeström *et al.*⁵⁸. These AuNPs were functionalized with a covalently linked (11-mercaptoundecyl)-*N,N,N*-trimethylammonium bromide (MUTAB) ligand, which due to a quaternary ammonium group gives the AuNPs a cationic surface over a wide range of pH values. The small AuNPs with a D_{core} of 2.5 nm (characterized with TEM) were synthesized as previously described by Kostiaainen *et al.*⁵⁹ taking advantage of the biphasic Brust-Schiffrin method⁵¹⁰. These AuNPs were functionalized with an alkyl-oxyethylene ligand also containing a quaternary ammonium group.

3.1. Dynamic light scattering (DLS)

The hydrodynamic diameter, D_{H} , of the AuNPs was determined by dynamic light scattering (DLS) using a Zetasizer Nano ZS (Malvern Instruments Ltd) equipped with a He-Ne ion laser at a wavelength of 633 nm. The measurements were carried out at 25 °C, and the scattered light was detected at an angle of 173° (backscattering) with laser attenuation and measurement position adjusted automatically by the Malvern software. The D_{H} was estimated from the volume-based particle size distribution, and a refractive index of 0.18 and an absorption index of 3.4 were used for the distribution estimation.⁵⁷ The measurements were done in disposable Brand® micro UV-cuvettes, and each sample consisted of 100 µL of neutral aqueous AuNP solution ($2\text{--}17 \times 10^{12}$ AuNPs/mL). Three

measurements of 10 runs with 5 s duration was performed for each sample, and the D_H was obtained as the average of at least three samples with different concentrations.

The zeta potential, ζ , of the small AuNP was determined as previously described by Kostianen *et al.*⁵⁹, whereas the zeta potential of the two larger AuNPs was determined using a Zetasizer Nano ZS90 (Malvern Instruments Ltd) equipped with a 4 mV He-Ne ion laser at a wavelength of 633 nm and an Avalanche photodiode detector at an angle of 90°. The measurements were carried out at 25 °C and the zeta potential was estimated using the Smoluchowski model. The measurements were done in disposable PMMA macro cuvettes using a ZEN1002 dip cell, and each sample consisted of 800 μ L of neutral aqueous AuNP solution (8×10^{12} AuNPs/mL). Six measurements of 30 runs were performed for each sample, and the zeta potential was obtained as the average of these measurements.

The hydrodynamic diameter, D_H , and the zeta potential, ζ , of the AuNPs are presented in Table S1. The DLS measurements also indicated that the AuNPs had a narrow size distribution and that there were nearly no aggregation of the AuNPs.

Table S1. Properties of the cationic AuNPs.

	D_{core} [nm]	D_H [nm]	ζ [mV]
Small AuNP	2.5	8.5	5.0
Large AuNP	10.9	14.7	34.0
Extra-large AuNP	12.4	15.8	36.5

3.2. AuNP colloidal stability

The colloidal stability of the AuNPs in solutions of different ionic strength was studied using UV/Vis spectroscopy. The colloidal stability of the AuNPs was determined as a function of ionic strength by the aggregation index $A_{800 \text{ nm}} / A_{522 \text{ nm}}$, where $A_{800 \text{ nm}}$ and $A_{522 \text{ nm}}$ are the absorbances at a wavelength of 800 nm respective 522 nm measured by UV/Vis spectroscopy. The UV/Vis spectra were recorded at 2 nm intervals in the wavelength range of 400 nm to 850 nm using a BioTek Cytation 3 Microplate reader. The measurements were done at 37 °C from 96-well polystyrene microplates without a lid (Thermo Scientific™ Nunc™ MicroWell™), and a sample size of 70 μ L was used. For the measurements a series of samples with different NaCl concentration was prepared, but the AuNP concentration was kept constant at 6 nM ($D_{\text{core}} = 10.9 \text{ nm}$ or $D_{\text{core}} = 12.4 \text{ nm}$) or at 260 nM ($D_{\text{core}} = 2.5 \text{ nm}$) for all samples.

For the small AuNPs ($D_{\text{core}} = 2.5 \text{ nm}$), the aggregation index did not remarkably change with increasing NaCl concentrations, indicating that the small AuNPs maintain good colloidal stability even at high NaCl concentrations (Fig. S4a). The large AuNPs ($D_{\text{core}} = 10.9 \text{ nm}$) were observed to aggregate at an ionic strength, c_{NaCl} , of about 750 mM (Fig. S4b), whereas the extra-large AuNPs ($D_{\text{core}} = 12.4 \text{ nm}$) aggregated at $c_{\text{NaCl}} \sim 500 \text{ mM}$ (Fig. S4c). The aggregation is reversible, and the AuNPs will detach from each other if the ionic strength is decreased. Similar behavior have been demonstrated also before for like-charged AuNPs.^{S11}

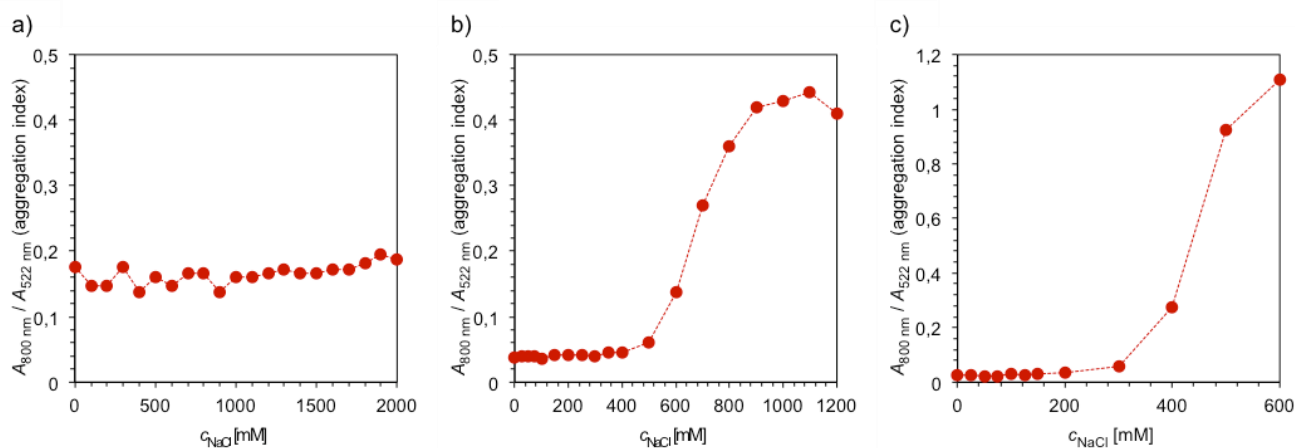


Fig. S4. Colloidal stability of the AuNPs measured by UV/Vis spectroscopy. The colloidal stability is determined as a function of the ionic strength by the aggregation index ($A_{800\text{nm}} / A_{522\text{nm}}$). a) Small AuNPs ($D_{\text{core}} = 2.5$ nm). b) Large AuNPs ($D_{\text{core}} = 10.9$ nm). c) Extra-large AuNPs ($D_{\text{core}} = 12.4$ nm).

4. AuNP binding properties of DNA origami structures

The electrostatic binding of the cationic AuNPs to the negatively charged DNA origami structures was studied with agarose gel electrophoretic mobility shift assay (EMSA) and UV/Vis spectroscopy.

4.1. Electrophoretic mobility shift assay (EMSA)

For the electrophoretic mobility shift assay (EMSA) a 2 % (w/v) agarose gel was prepared as earlier described in Section 2.6. The DNA origami concentration was kept constant at 5.0 nM in all samples, but the AuNP concentration varied between the samples to obtain the desired $n_{\text{AuNP}}/n_{\text{origami}}$ (amount of AuNPs / amount of DNA origamis) ratio. PEG-purified DNA origami solution was mixed with AuNP solution and incubated for 20-30 minutes at room temperature to allow the formation of complexes. The samples were not dialyzed against decreasing ionic strength and hence the NaCl concentration was 0 mM for the 6HB and 24HB samples and 2 mM for the 60HB samples (The 60HB folding buffer contains 5 mM NaCl, therefore also the EMSA samples contain small amounts of NaCl). After the incubation, 4 μL of Gel Loading Dye Blue (6 $\times$) was added to the sample (volume of 20 μL), and the whole sample of 24 μL was loaded into the gel well. The gel electrophoresis was performed in an ice bath at a constant voltage of 95 V for 45 minutes using a BioRad PowerPac™ Basic. 1 $\times$ TAE buffer containing 11 mM MgCl_2 was used as running buffer. The gel was visualized under UV light using a BioRad Gel Doc™ Ez Imager.

4.2. UV/Vis spectroscopy

The electrostatic binding of the cationic AuNPs to the negatively charged DNA origami structures was studied in solutions of different ionic strength using UV/Vis spectroscopy. The colloidal stability of the AuNPs in DNA origami solutions of different concentration was determined as a function of ionic strength by the aggregation index $A_{800\text{ nm}} / A_{522\text{ nm}}$, where $A_{800\text{ nm}}$ and $A_{522\text{ nm}}$ are the absorbances at a wavelength of 800 nm respective 522 nm measured by UV/Vis spectroscopy. The UV/Vis spectra were recorded at 2 nm intervals in the wavelength range of 400 nm to 850 nm using a BioTek Cytation 3 Microplate reader. The measurements were done at 37 °C from 96-well polystyrene microplates without a lid (Thermo Scientific™ Nunc™ MicroWell™), and a sample size of 70 µL was used. For the measurements a series of samples with different DNA origami concentration and different NaCl concentration was prepared, but the AuNP concentration was kept constant at 6 nM ($D_{\text{core}} = 10.9\text{ nm}$ or $D_{\text{core}} = 12.4\text{ nm}$) or at 260 nM ($D_{\text{core}} = 2.5\text{ nm}$) for all samples.

4.2.1. Additional data

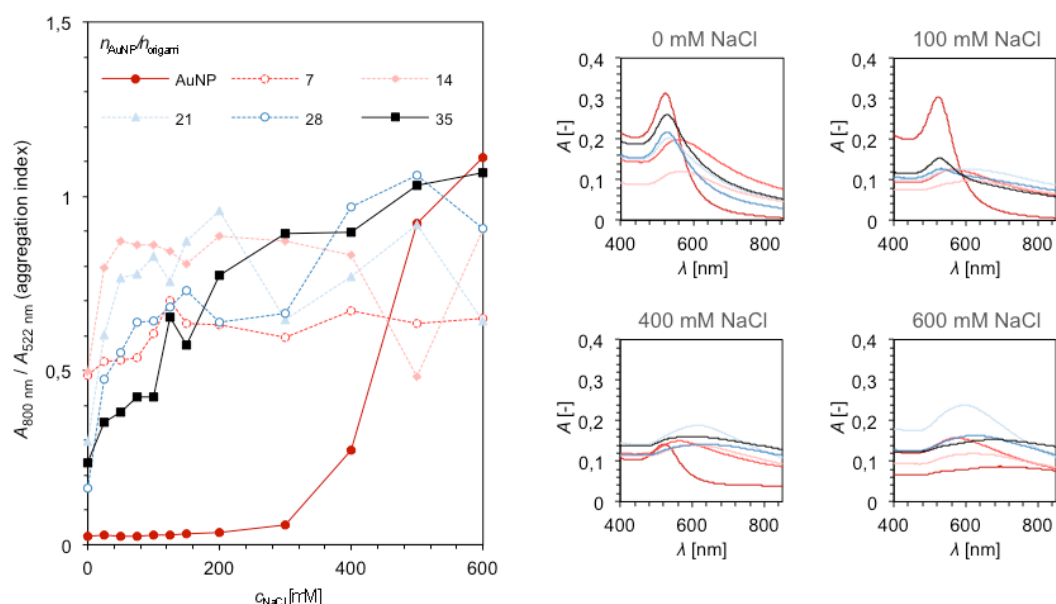


Fig. S5. Colloidal stability of extra-large AuNPs ($D_{\text{core}} = 12.4\text{ nm}$) when mixed with increasing amounts of the 6HB. The colloidal stability in the left graph is determined by UV/Vis spectroscopy as a function of ionic strength by the aggregation index, $A_{800\text{ nm}} / A_{522\text{ nm}}$. The small graphs to the right show the whole absorbance spectra at selected ionic strengths. The stoichiometric ratios between AuNPs and DNA origami structures ($n_{\text{AuNP}}/n_{\text{origami}}$) and the corresponding colors are the same in the spectra as in the left graph.

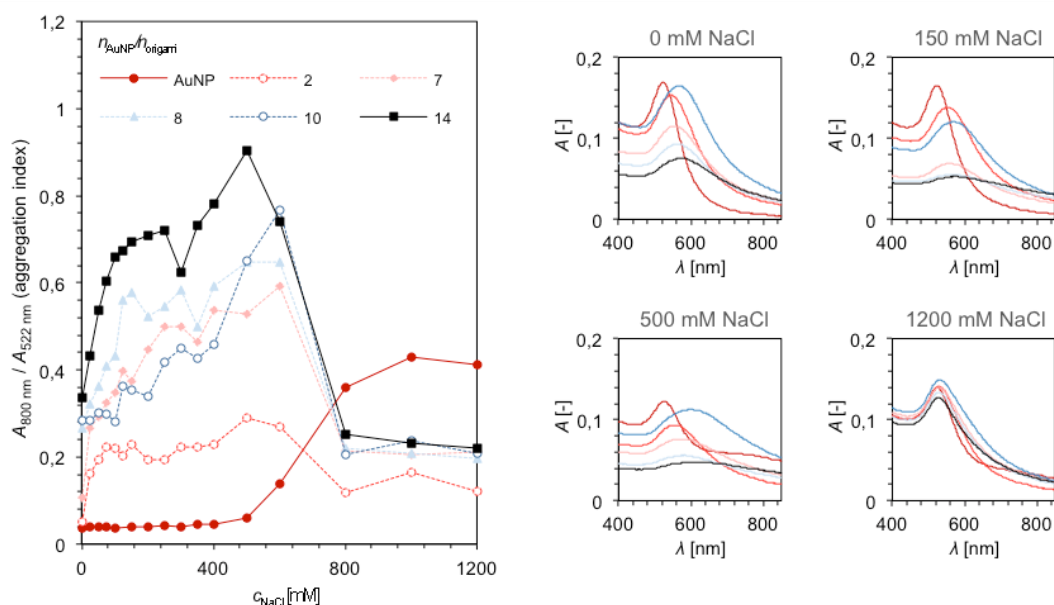


Fig. S6. Colloidal stability of large AuNPs ($D_{\text{core}} = 10.9$ nm) when mixed with increasing amounts of the 24HB. The colloidal stability in the left graph is determined by UV/Vis spectroscopy as a function of ionic strength by the aggregation index, $A_{800 \text{ nm}} / A_{522 \text{ nm}}$. The small graphs to the right show the whole absorbance spectra at selected ionic strengths. The stoichiometric ratios between AuNPs and DNA origami structures ($n_{\text{AuNP}}/n_{\text{origami}}$) and the corresponding colors are the same in the spectra as in the left graph.

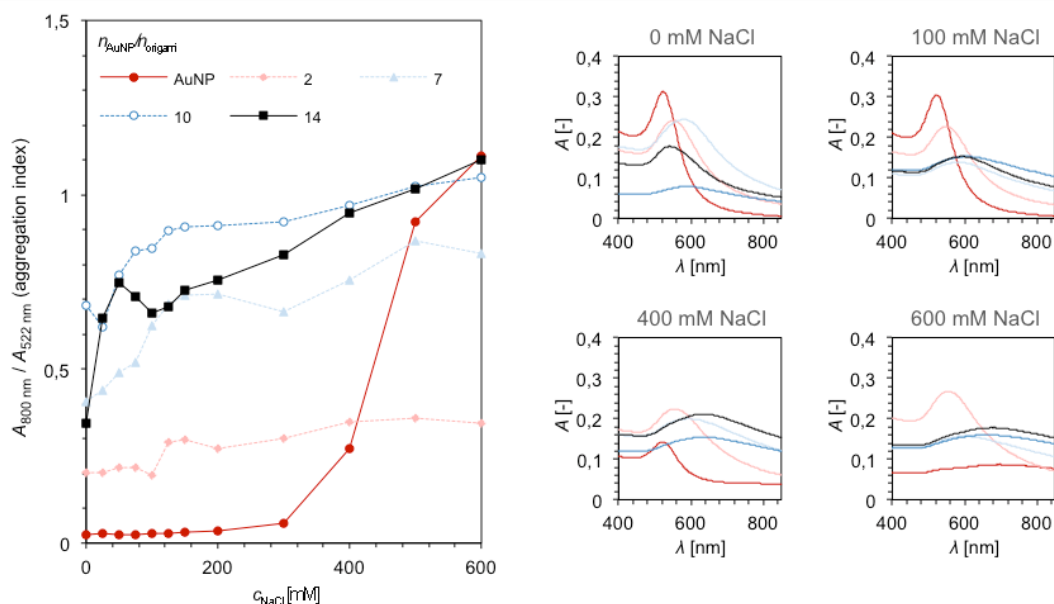


Fig. S7. Colloidal stability of extra-large AuNPs ($D_{\text{core}} = 12.4$ nm) when mixed with increasing amounts of the 24HB. The colloidal stability in the left graph is determined by UV/Vis spectroscopy as a function of ionic strength by the aggregation index, $A_{800 \text{ nm}} / A_{522 \text{ nm}}$. The small graphs to the right show the whole absorbance spectra at selected ionic strengths. The stoichiometric ratios between AuNPs and DNA origami structures ($n_{\text{AuNP}}/n_{\text{origami}}$) and the corresponding colors are the same in the spectra as in the left graph.

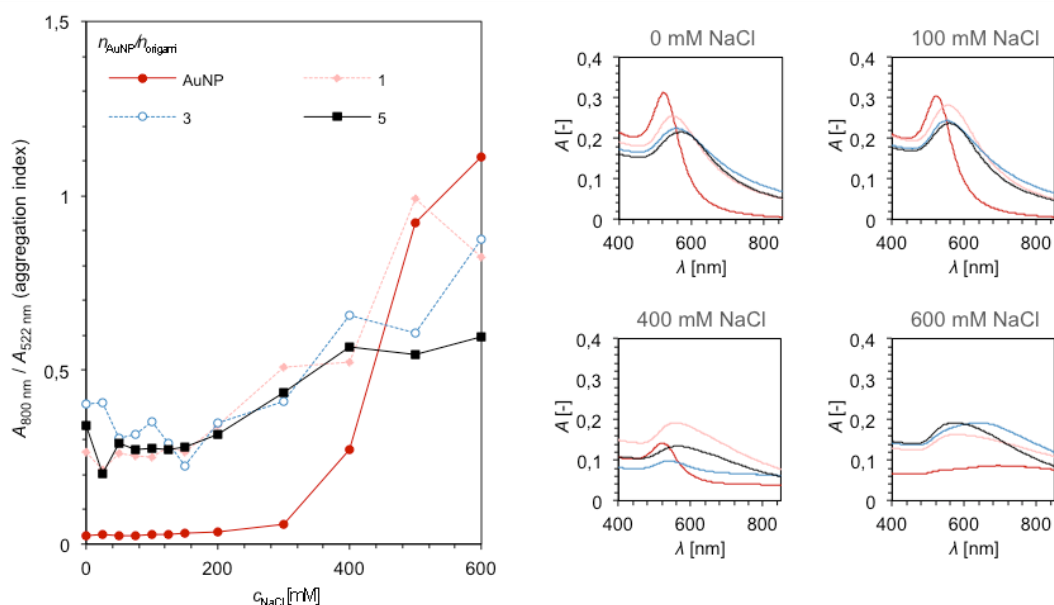


Fig. S8. Colloidal stability of extra-large AuNPs ($D_{\text{core}} = 12.4$ nm) when mixed with increasing amounts of the 60HB. The colloidal stability in the left graph is determined by UV/Vis spectroscopy as a function of ionic strength by the aggregation index, $A_{800 \text{ nm}} / A_{522 \text{ nm}}$. The small graphs to the right show the whole absorbance spectra at selected ionic strengths. The stoichiometric ratios between AuNPs and DNA origami structures ($n_{\text{AuNP}}/n_{\text{origami}}$) and the corresponding colors are the same in the spectra as in the left graph.

5. Electrostatic self-assembly of DNA origami and AuNP

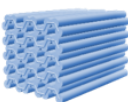
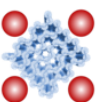
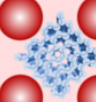
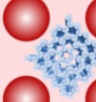
The electrostatic self-assembly of negatively charged DNA origami structures and cationic AuNPs was studied using three different DNA origami shapes (6HB, 24HB and 60HB) and cationic AuNPs of three different sizes. Table S1 was used to theoretically predict which combinations are feasible and only these combinations were tested experimentally under different conditions. Rod-like structures are prone to form two-dimensional (2D) lattices with either a hexagonal or square lattice packing and the initial prediction of successful combinations was therefore done based on these two types of packing.

On the basis of the theoretical calculations, seven combinations of DNA origami and AuNPs were tested experimentally under different conditions (colored background in table S1). Out of these tested combinations, only the combination of 6HB and small AuNPs ($D_{\text{H}} = 8.5$ nm) yielded highly ordered superlattices (green background in table S1), which demonstrate that in addition to shape complementarity also charge complementarity between the building blocks is important for crystal formation.

The combination of 24HB and extra-large AuNPs ($D_{\text{H}} = 15.8$ nm) as well as the combination of 60HB and small AuNPs ($D_{\text{H}} = 8.5$ nm) were not tested experimentally since they were considered unfeasible (white background in table S1). The 24HB should be optimal for formation of 2D square lattices with all three kinds of AuNPs, but its short length was observed to be problematic for lattice formation with both small AuNPs ($D_{\text{H}} = 8.5$ nm) and large AuNPs ($D_{\text{H}} = 14.7$ nm). Since the length of 100 nm was not enough to guide the directional growth of the lattice for neither of the smaller AuNPs, it was predicted that this will be the case also for the

extra-large AuNPs ($D_H = 15.8$ nm). For the combination of 60HB and small AuNPs ($D_H = 8.5$ nm), neither a hexagonal nor a square lattice packing is possible and therefore this combination was not tested experimentally.

Table S2. Theoretical predictions for electrostatic self-assembly of DNA origami and AuNPs. For a 2D AuNP-DNA origami lattice, the geometry is dependent on the size ratio of the diameter of the building blocks. In the calculations, the diameter for the 60HB structure is approximated by the diagonal of the cross-section. Green tick (✓) indicates theoretically feasible structure, red cross (✗) indicates theoretically unfeasible structure and orange question mark (?) that both alternatives are in theory feasible. Green background marks an experimentally successful combination of DNA origami and AuNPs, whereas red background marks an experimentally tested, unsuccessful combination. The combinations with white background have not been experimentally tested.

	Small AuNPs $(D_H = 8.5 \text{ nm})$ 		Large AuNPs $(D_H = 14.7 \text{ nm})$ 		Extra-large AuNPs $(D_H = 15.8 \text{ nm})$ 	
6HB $(D_{6HB} = 6 \text{ nm})$ 	$D_{6HB}/D_{AuNP} = 0.71$		$D_{6HB}/D_{AuNP} = 0.41$		$D_{6HB}/D_{AuNP} = 0.38$	
	Hexagonal ✗ 	Square ✓ 	Hexagonal ? 	Square ? 	Hexagonal ✓ 	Square ✗ 
24HB $(D_{24HB} = 16 \text{ nm})$ 	$D_{AuNP}/D_{24HB} = 0.53$		$D_{AuNP}/D_{24HB} = 0.92$		$D_{AuNP}/D_{24HB} = 0.99$	
	Hexagonal ✗ 	Square ✓ 	Hexagonal ✗ 	Square ✓ 	Hexagonal ✗ 	Square ✓ 
60HB $(D_{60HB} = 28.3 \text{ nm})$ 	$D_{AuNP}/D_{60HB} = 0.30$		$D_{AuNP}/D_{60HB} = 0.52$		$D_{AuNP}/D_{60HB} = 0.55$	
	Hexagonal ✗ 	Square ✗ 	Hexagonal ✗ 	Square ✓ 	Hexagonal ✗ 	Square ✓ 

Hexagonal

$D_{AuNP}/D_{origami} = 0.15-0.41$

Square

$D_{AuNP}/D_{origami} = 0.41-1.00$

5.1. Formation of DNA origami-AuNP assemblies

The DNA origami-AuNP assemblies were formed during dialysis with floating dialysis cups (Slide-A-Lyzer® MINI Dialysis Units, 3500 MVCO, Thermo Scientific) that floated on the dialysate during continuous stirring. The dialysate consisted of 5 mM $MgCl_2$ and a specific $NaCl$ concentration (c_{NaCl}). Depending on the size of the AuNP, the c_{NaCl} was 500-750 mM in the beginning of the dialysis, and the c_{NaCl} was decreased by 50 mM every 30 minutes until a final c_{NaCl} of 0 mM was reached. The dialysate was then changed one more time (to $c_{NaCl} = 0$ mM and $c_{MgCl_2} = 5$ mM) and the dialysis was continued for two hours. Note: before the sample was placed

into the dialysis cup, the DNA origami solution and the AuNP solution were mixed, and the NaCl concentration of the mixture adjusted to the initial c_{NaCl} of the dialysate.

6. Small-angle X-ray scattering (SAXS)

The formed DNA origami-AuNP assemblies were studied with small-angle X-ray scattering (SAXS) in order to gain information about their structural geometries. For the SAXS measurements, 10 μL of the aqueous sample from dialysis was placed in a metal ring and tightly sealed by Kapton tape on both sides, resulting in a sample with a liquid thickness of approximately 0.9 mm. To reduce background scattering from air, vacuum was applied in the sample environment. The measurements were carried out using a Bruker Microstar microfocus rotating anode X-ray source (Cu K α radiation, $\lambda = 1.54 \text{ \AA}$). The X-ray beam was monochromated and focused by a Montel multilayer focusing monochromator (Incotec). The beam was further collimated with four sets of collimation slits (JJ X-ray) resulting in a final spot size of approximately 1 mm in diameter at the sample position. The scattered intensity was collected using a Hi-Star 2D area detector (Bruker). Depending on the sample, the sample-to-detector distance was 0.59 m and/or 1.59 m, and a silver behenate standard sample was used for the calibration of the length of the scattering vector q . One-dimensional SAXS data was obtained by azimuthally averaging the 2D scattering data, and the magnitude of the scattering vector q is given by

$$q = 4\pi \sin\theta / \lambda$$

where 2θ is the scattering angle and λ the wavelength of the radiation (in this case 1.54 $\text{\AA}$). The measured structure factor, $S(q)$, was obtained by dividing the scattering intensity $I(q)$ by the $I(q)$ measured from AuNPs in solution using the local monodisperse approximation,^{S12} whereas the theoretical $S(q)$ was calculated using PowderCell^{S13}.

6.1. Additional SAXS data

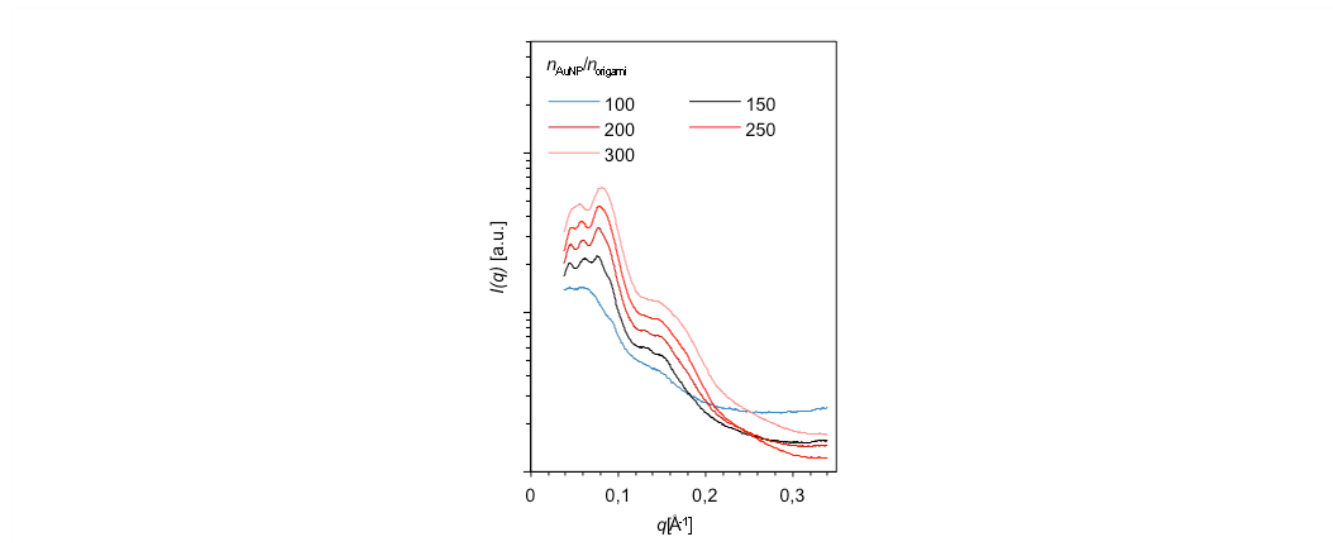


Fig. S9. SAXS data measured from samples having different stoichiometric ratios between small AuNPs ($D_{\text{core}} = 2.5$ nm) and the 6HB ($n_{\text{AuNP}}/n_{\text{origami}}$). The sample-to-detector distance is 0.59 m.

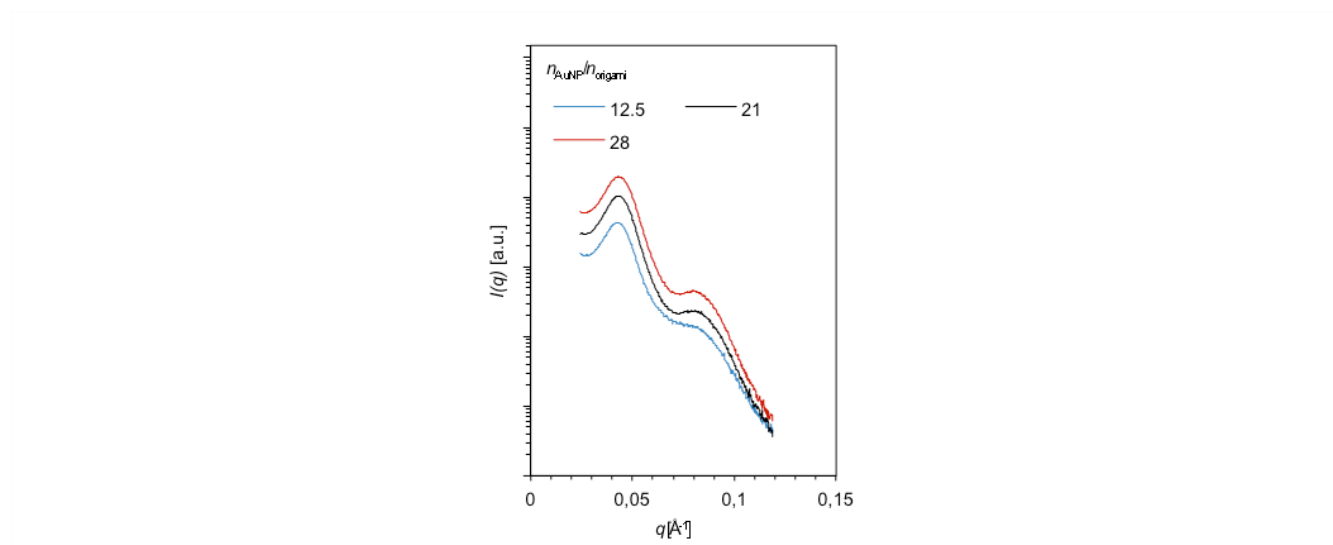


Fig. S10. SAXS data measured from samples having different stoichiometric ratios between extra-large AuNPs ($D_{\text{core}} = 12.4$ nm) and the 6HB ($n_{\text{AuNP}}/n_{\text{origami}}$). The sample-to-detector distance is 1.59 m.

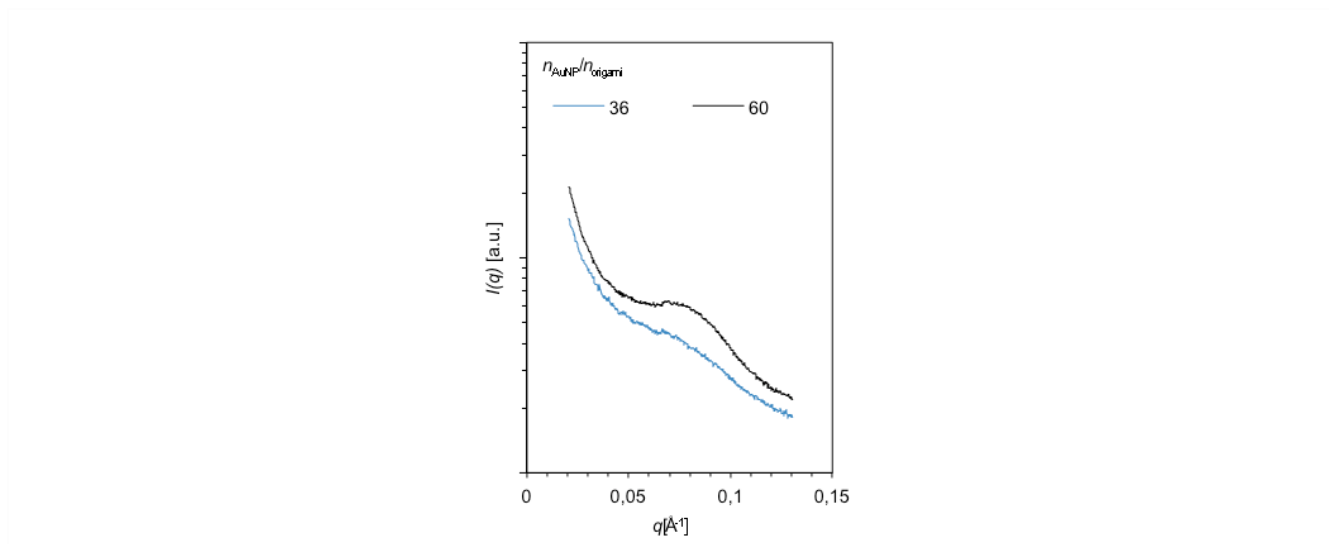


Fig. S11. SAXS data measured from samples having different stoichiometric ratios between small AuNPs ($D_{\text{core}} = 2.5$ nm) and the 24HB ($n_{\text{AuNP}}/n_{\text{origami}}$). The sample-to-detector distance is 1.59 m.

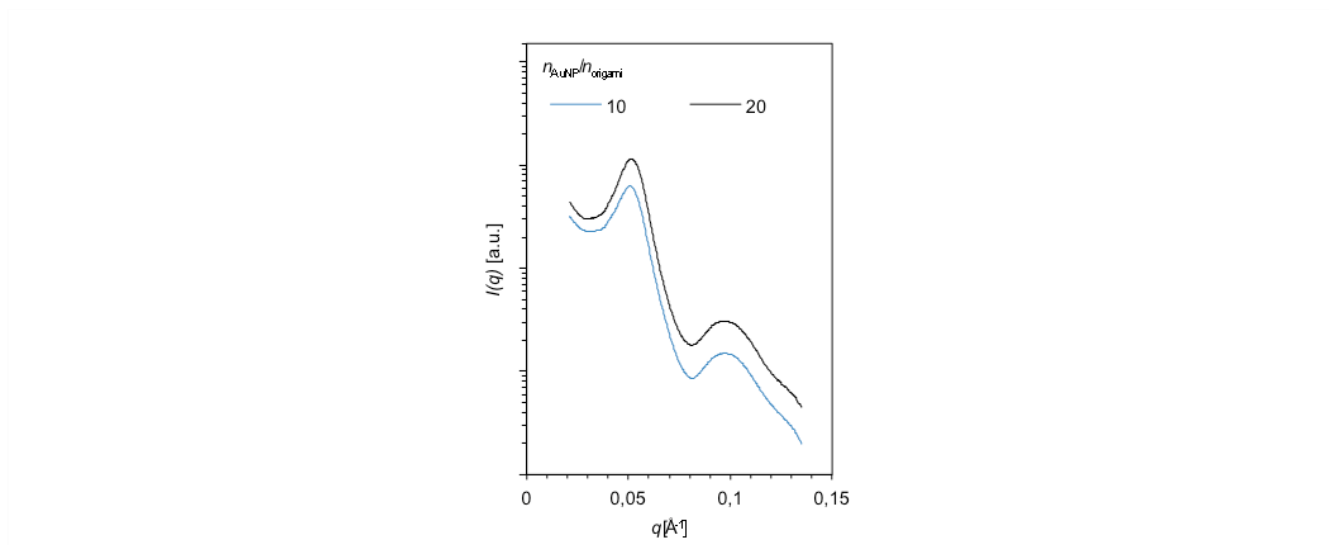


Fig. S12. SAXS data measured from samples having different stoichiometric ratios between large AuNPs ($D_{\text{core}} = 10.9$ nm) and the 24HB ($n_{\text{AuNP}}/n_{\text{origami}}$). The sample-to-detector distance is 1.59 m.

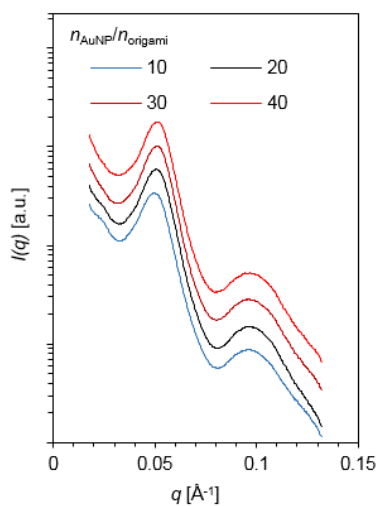


Fig. S13. SAXS data measured from samples having different stoichiometric ratios between large AuNPs ($D_{\text{core}} = 10.9$ nm) and the 60HB ($n_{\text{AuNP}}/n_{\text{origami}}$). The sample-to-detector distance is 1.59 m.

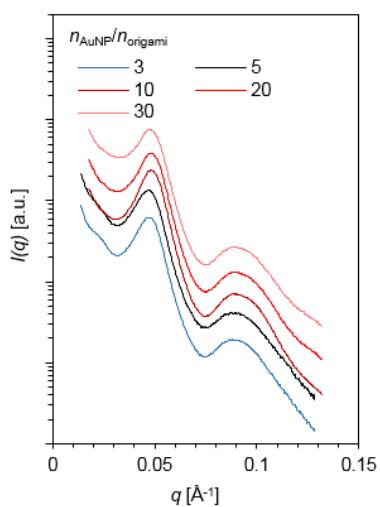


Fig. S14. SAXS data measured from samples having different stoichiometric ratios between extra-large AuNPs ($D_{\text{core}} = 12.4$ nm) and the 60HB ($n_{\text{AuNP}}/n_{\text{origami}}$). The sample-to-detector distance is 1.59 m.

7. Imaging of assembled structures

The DNA origami-AuNP assemblies were imaged using both conventional transmission electron microscopy and cryogenic transmission electron microscopy. Further, cryogenic electron tomography was performed to visualize the DNA origami-AuNP assemblies from different orientations.

7.1. Conventional transmission electron microscopy (TEM)

The conventional transmission electron microscopy (TEM) images were obtained using a FEI Tecnai 12 Bio-Twin instrument operated at an acceleration voltage of 120 kV. The samples were prepared by applying 3 μ L of dialyzed sample solution on plasma cleaned (30 seconds oxygen plasma flash) Formvar carbon coated copper grids (Electron Microscopy Science). The samples were incubated for 2.5 minutes before excess sample solution was blotted away with filter paper. The samples were left to dry under ambient conditions for at least one hour before imaging.

7.2. Cryogenic transmission electron microscopy (cryo-TEM) and electron tomography (cryo-ET)

The cryogenic transmission electron microscopy (cryo-TEM) images were obtained using a JEM 3200FSC field emission cryo-TEM (JEOL) operated at 300 kV in bright field mode with an Omega type Zero-loss energy filter. The images were acquired with Gatan Digital Micrograph software while the specimen temperature was maintained at -187 °C. The cryo-TEM samples were prepared by applying 3 μ L of dialyzed sample solution (diluted 1:5 or 1:10 in distilled H₂O) on plasma cleaned (30 seconds oxygen plasma flash) 300-mesh copper grids with lacey carbon support film (Ted Pella, Inc.) and plunge freezed in 1:1 liquid propane/ethane mixture using FEI Vitrobot™ with 2.5 s blotting time under 55 % humidity.

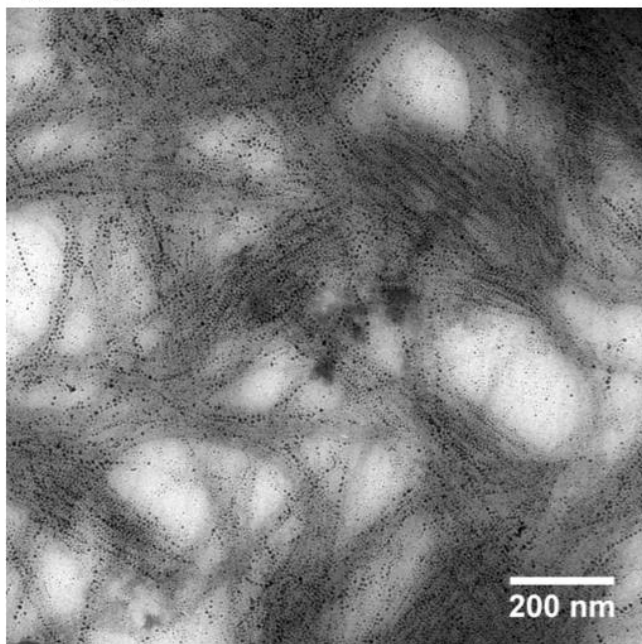
Electron tomography tilt series were acquired with the SerialEM-software package^{S14}. Samples were tilted between $\pm 69^\circ$ angles with 2-3° increment steps. Prealignment, fine alignment and the cropping of the tilt series was executed with IMOD^{S15}. The images were binned 2-4 times to reduce noise and computation time. Maximum entropy method (MEM) reconstruction scheme was carried out with custom made program on Mac or Linux cluster with regularization parameter value of $\lambda = 0.001$ ^{S16}. UCSF Chimera package was used for the volumetric graphics and analyses.

7.3. Additional images of assembled structures

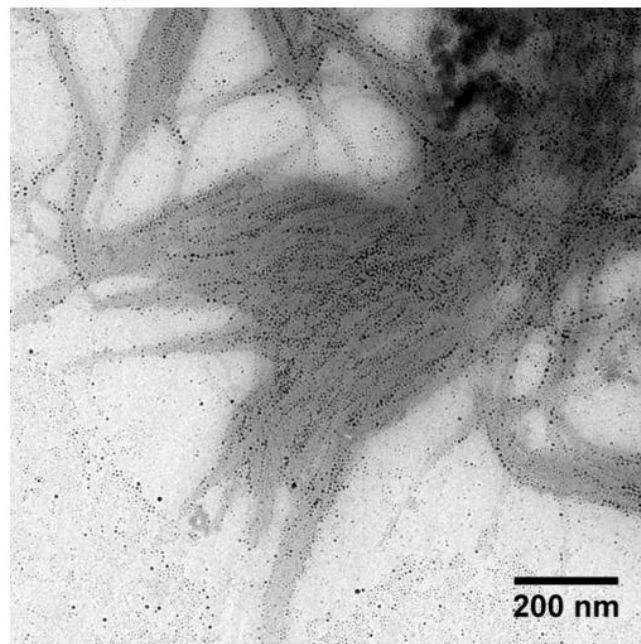
7.3.1. 6HB + small AuNPs ($D_{\text{core}} = 2.5 \text{ nm}$)

Electrostatic self-assembly of the 6HB and small AuNPs ($D_{\text{core}} = 2.5 \text{ nm}$) yielded well-ordered crystalline structures for a wide range of stoichiometric ratios ($n_{\text{AuNP}}/n_{\text{origami}}$). Below, collections of TEM and cryo-TEM images for samples having different $n_{\text{AuNP}}/n_{\text{origami}}$ are shown.

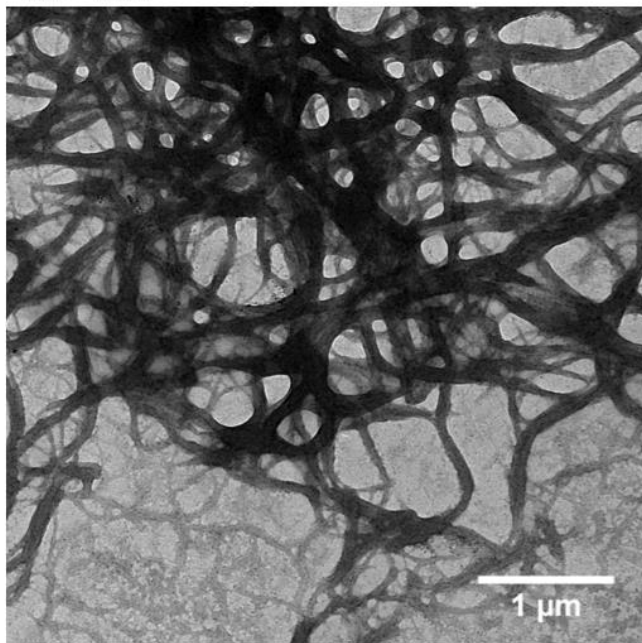
$n_{\text{AuNP}}/n_{\text{origami}} \sim 50$



$n_{\text{AuNP}}/n_{\text{origami}} \sim 50$



$n_{\text{AuNP}}/n_{\text{origami}} \sim 100$



$n_{\text{AuNP}}/n_{\text{origami}} \sim 100$

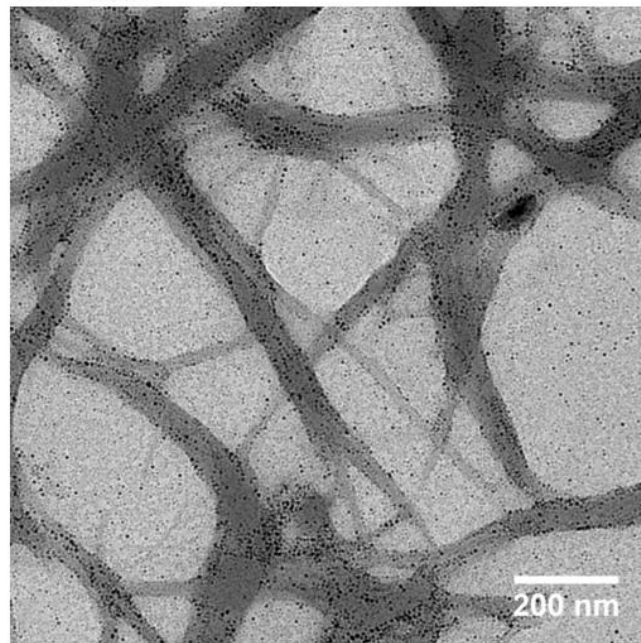
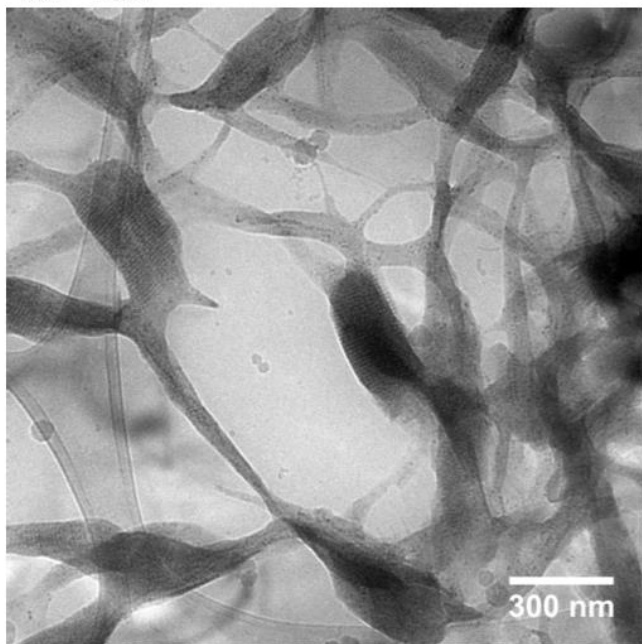
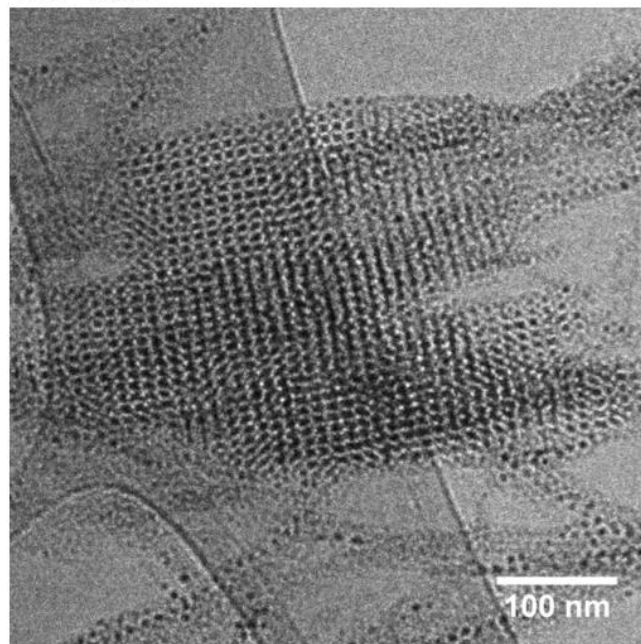


Fig. S15. TEM images of samples with excess of 6HB structures. The stoichiometric ratio between small AuNPs ($D_{\text{core}} = 2.5$ nm) and the 6HB is given as $n_{\text{AuNP}}/n_{\text{origami}}$.

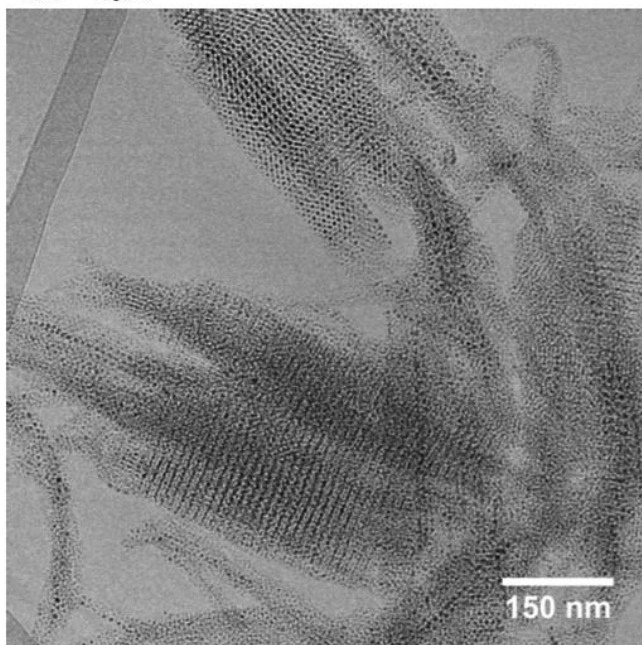
$n_{\text{AuNP}}/n_{\text{origami}} \sim 150$



$n_{\text{AuNP}}/n_{\text{origami}} \sim 150$



$n_{\text{AuNP}}/n_{\text{origami}} \sim 150$



$n_{\text{AuNP}}/n_{\text{origami}} \sim 150$

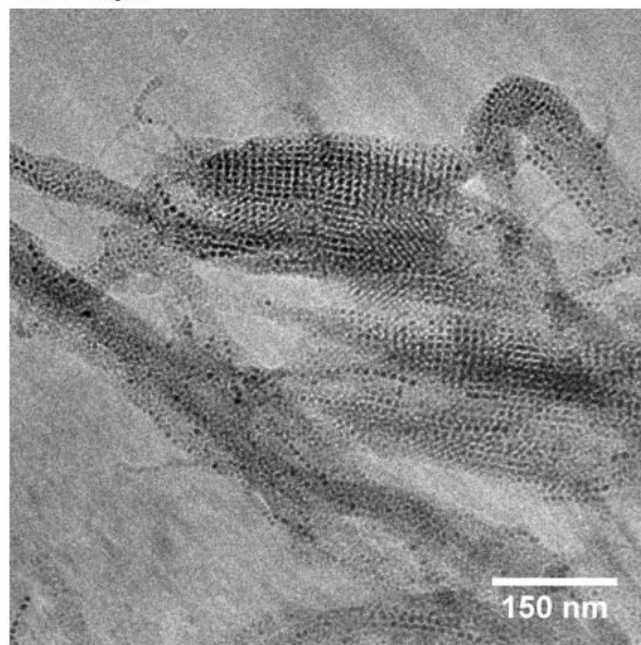
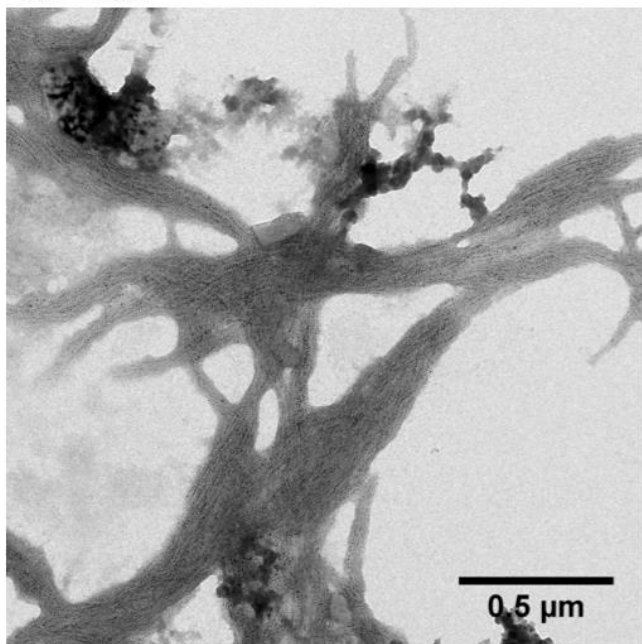
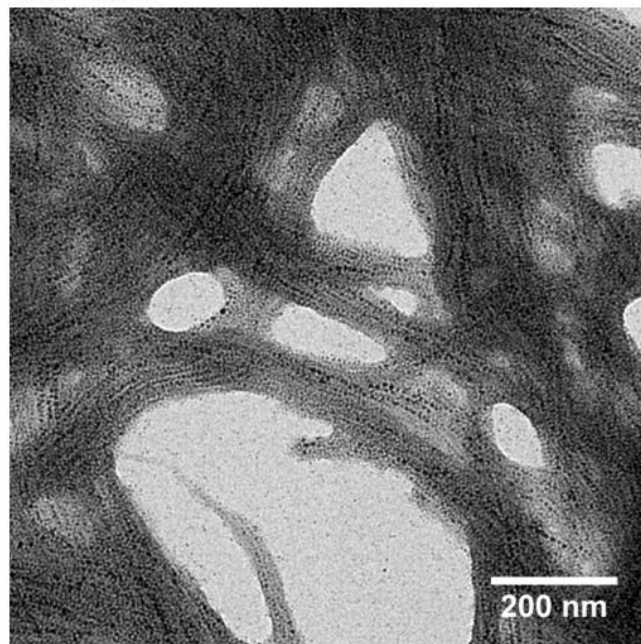


Fig. S16. Cryo-TEM images of samples with optimal stoichiometric ratio between small AuNPs ($D_{\text{core}} = 2.5$ nm) and the 6HB ($n_{\text{AuNP}}/n_{\text{origami}} = 150$).

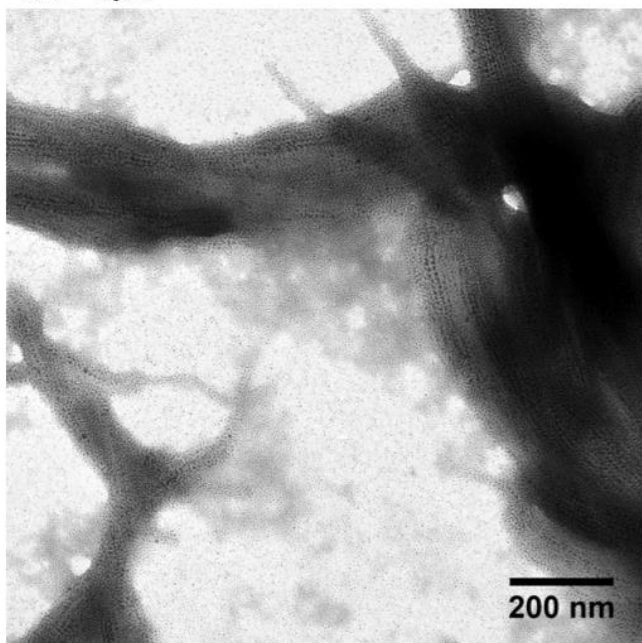
$n_{\text{AuNP}}/n_{\text{origami}} \sim 250$



$n_{\text{AuNP}}/n_{\text{origami}} \sim 250$



$n_{\text{AuNP}}/n_{\text{origami}} \sim 500$



$n_{\text{AuNP}}/n_{\text{origami}} \sim 500$

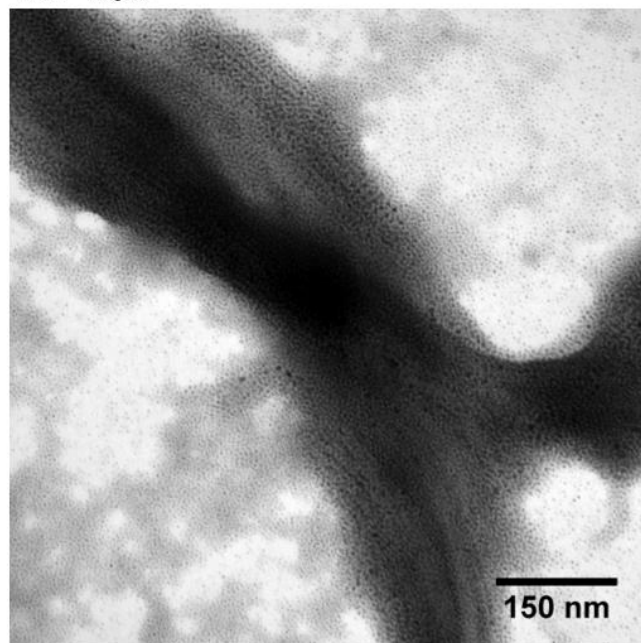


Fig. S17. TEM images of samples with excess of AuNPs. The stoichiometric ratio between small AuNPs ($D_{\text{core}} = 2.5$ nm) and the 6HB is given as $n_{\text{AuNP}}/n_{\text{origami}}$.

7.3.2. 6HB + large AuNPs ($D_{\text{core}} = 10.9 \text{ nm}$)

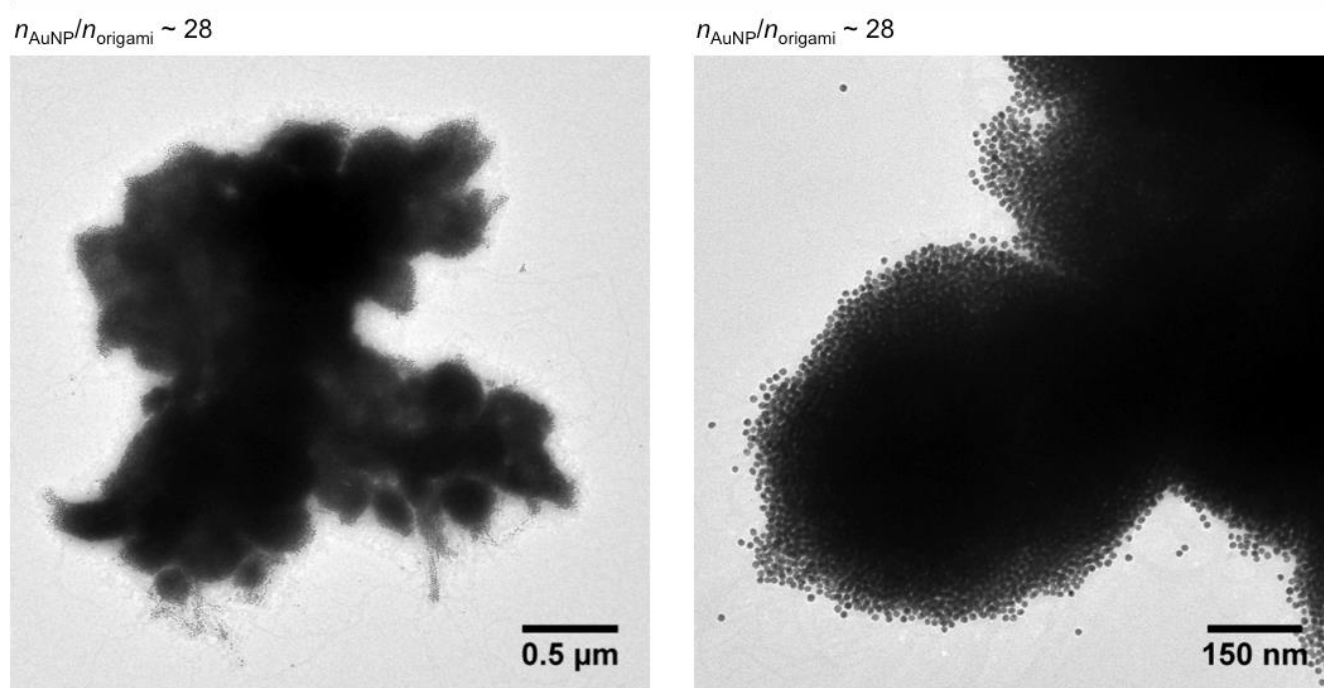


Fig. S18. TEM images of samples having a stoichiometric ratio of 28 between large AuNPs ($D_{\text{core}} = 10.9 \text{ nm}$) and the 6HB ($n_{\text{AuNP}}/n_{\text{origami}}$). As can be seen from the images, electrostatic self-assembly of the 6HB and large AuNPs yielded aggregates with only short range order.

7.3.3. 6HB + extra-large AuNPs ($D_{\text{core}} = 12.4 \text{ nm}$)

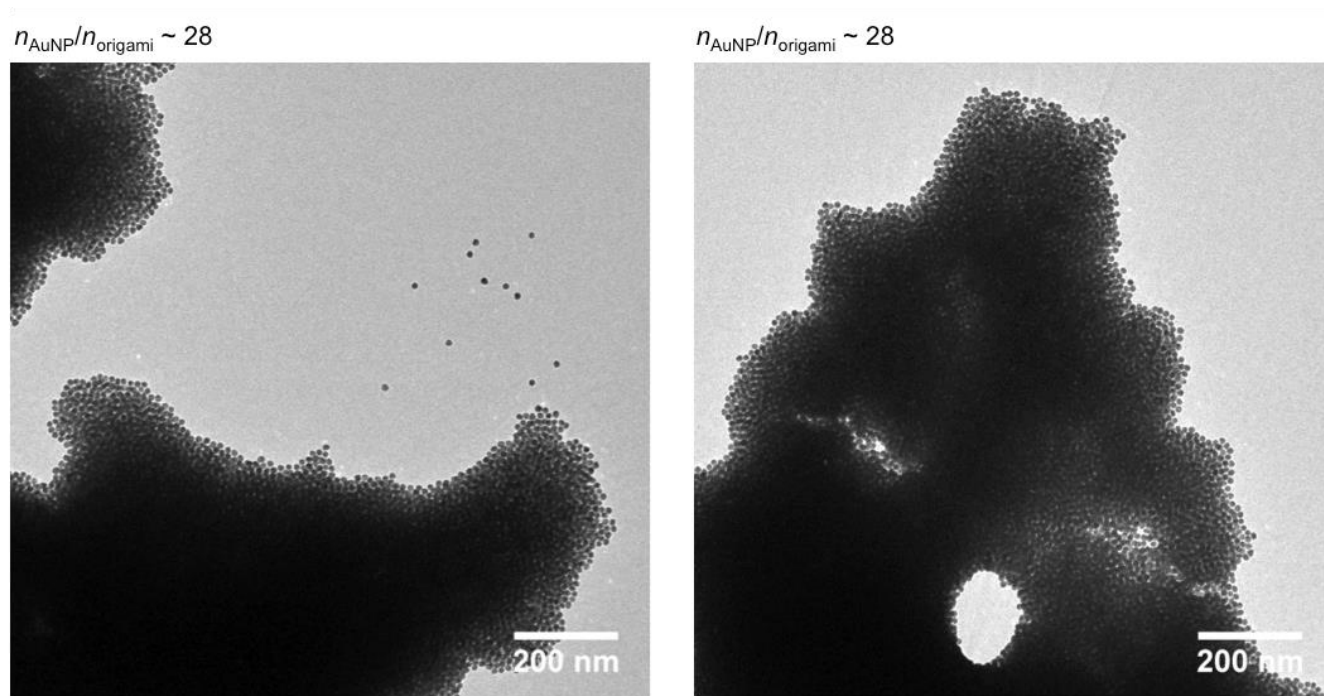


Fig. S19. TEM images of samples having a stoichiometric ratio of 28 between extra-large AuNPs ($D_{\text{core}} = 12.4 \text{ nm}$) and the 6HB ($n_{\text{AuNP}}/n_{\text{origami}}$). As can be seen from the images, electrostatic self-assembly of DNA origami 6HB structures and extra-large AuNPs yielded aggregates with only short range order.

7.3.4. 24HB + small AuNPs ($D_{\text{core}} = 2.5 \text{ nm}$)

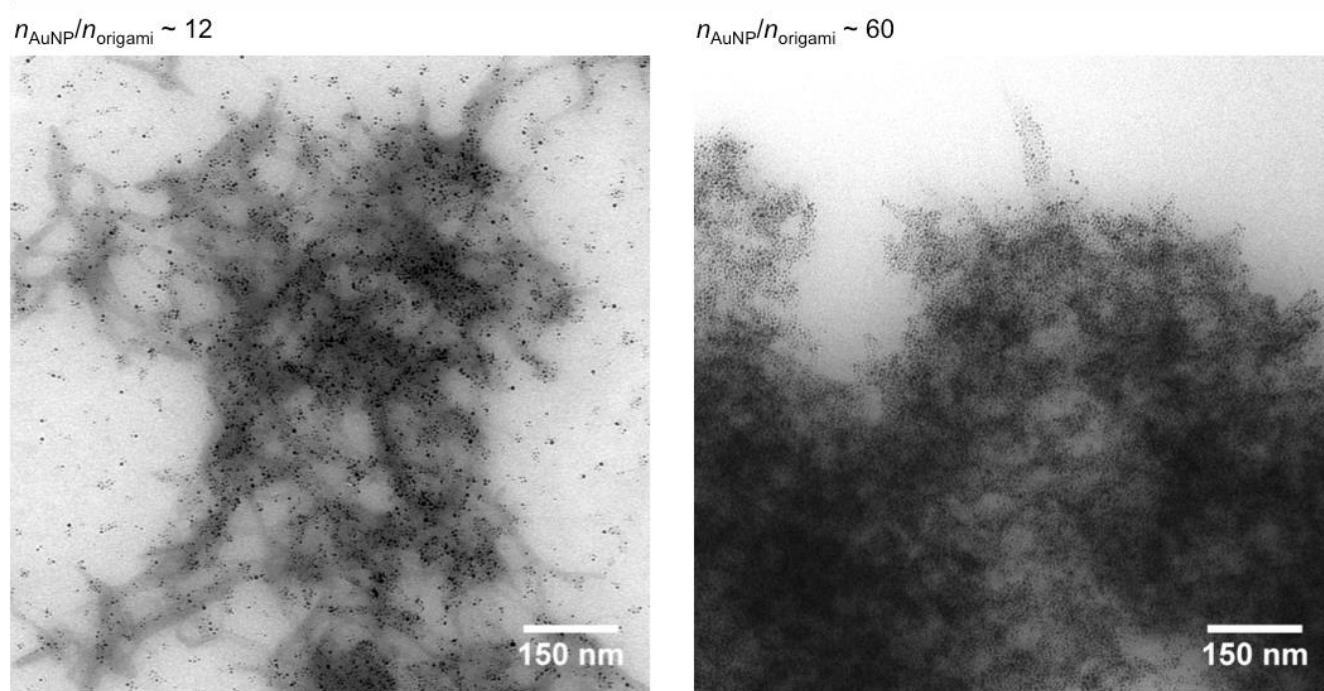


Fig. S20. TEM images of samples having different stoichiometric ratios between small AuNPs ($D_{\text{core}} = 2.5 \text{ nm}$) and the 24HB ($n_{\text{AuNP}}/n_{\text{origami}}$). As can be seen from the images, electrostatic self-assembly of the 24HB and small AuNPs yielded aggregates with only short range order.

7.3.5. 24HB + large AuNPs ($D_{\text{core}} = 10.9 \text{ nm}$)

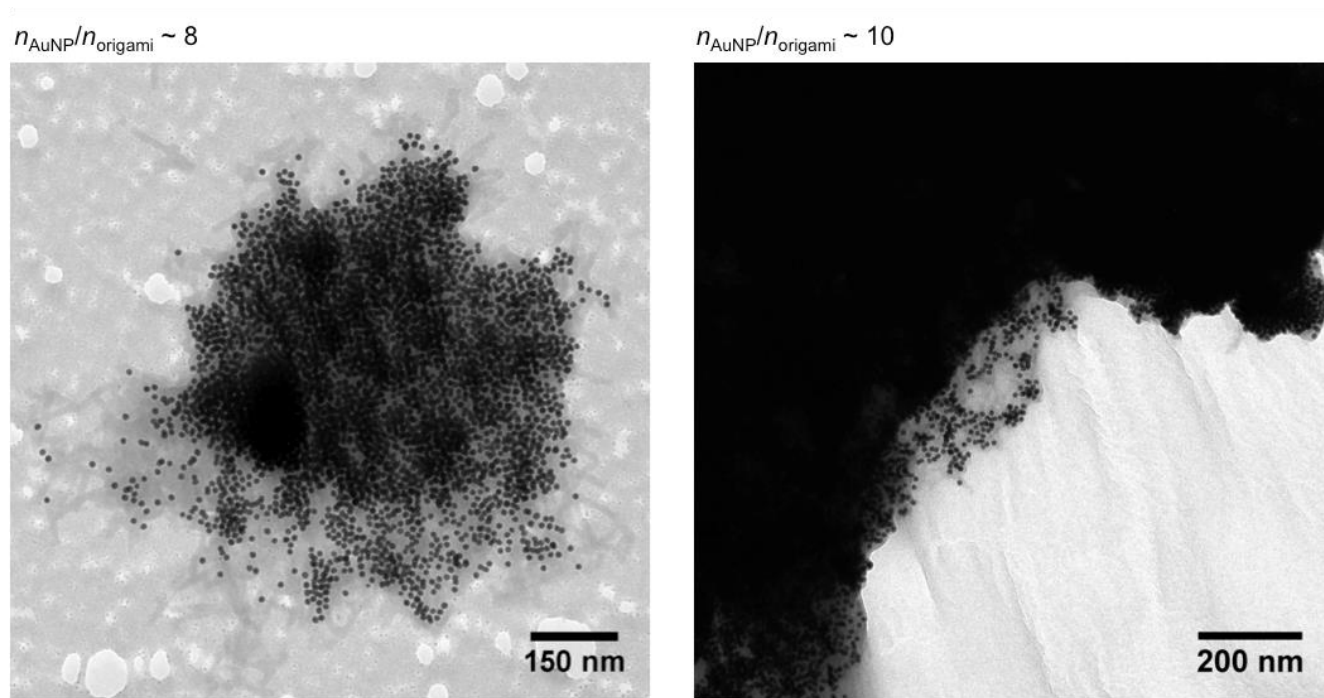


Fig. S21. TEM images of samples having different stoichiometric ratios between large AuNPs ($D_{\text{core}} = 10.9 \text{ nm}$) and the 24HB ($n_{\text{AuNP}}/n_{\text{origami}}$). As can be seen from the images, electrostatic self-assembly of the 24HB and large AuNPs yielded aggregates with only short range order.

7.3.6. 60HB + extra-large AuNPs ($D_{\text{core}} = 12.4 \text{ nm}$)

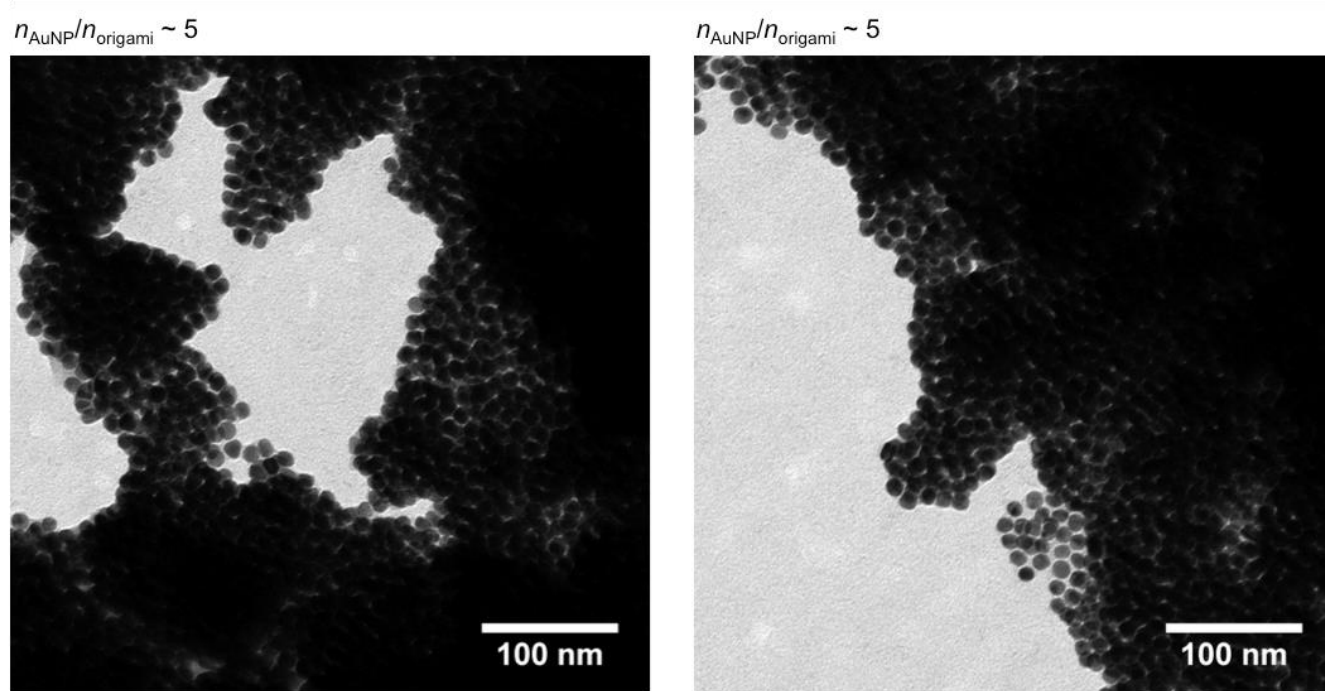


Fig. S22. TEM images of samples having a stoichiometric ratio of 5 between extra-large AuNPs ($D_{\text{core}} = 12.4 \text{ nm}$) and the 60HB ($n_{\text{AuNP}}/n_{\text{origami}}$). As can be seen from the images, electrostatic self-assembly of the 60HB and extra-large AuNPs yielded aggregates with only short range order.

8. Supporting information references

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