Growth rate and thermal properties of DNA origami filaments

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Figure S1. **Shape-complementary interfaces of the monomer.** The AB monomer displays A and B edges that are mutually complementary (a) and partially self-complementary (b and c) in shape. The A and B edges can be programmed to match perfectly, such to enable the attachment of consecutive monomers in a linear and periodic fashion (a). On the contrary, stacking at the A or B interfaces results in the head-to-head or tail-to-tail orientation of two monomers, leaving 1 bp-gap (dashed circles) between one (b) or two (c) helical patches. The DNA design maps (from caDNAno files) refer to the region at the interface of two monomers associated in one of three possible modes: AB, AA, and BB (scaffold sequence in grey, core staples in black and edge staples in blue and orange, for A and B tips, respectively). Close views of the 3D models of the monomer interfaces are also reported.
Figure S2. Details of the FRET-labelling strategy. Hybridization or stacking interactions between the monomers were monitored using reporter strands labelled at their termini with a FAM or TAMRA dye. Reporter strands are positioned at the edges of the monomer, either at the A or B tip, and are labelled such that – upon monomers association – the dyes are brought into close proximity, leading to a change in the FRET efficiency. Each type of monomer association, i.e. AB, AA or BB, is monitored using one of three possible FRET pairs (identified by different colors and index numbers). Specifically, the heterologous interaction at the AB interface (attainable either by hybridization or stacking) is monitored by the change in the FRET efficiency between the FAM$_1$-A/TAMRA$_1$-B dyes, located respectively at the helices 19 and 20 of the A and B tips (a). The isologous interaction at the A interface is visualized by the change in the FRET efficiency between the FAM$_2$-A/TAMRA$_2$-A dyes, located respectively at the helices 20 and 13 of the A tip (b). Positions were chosen to have maximal distance and still be located at perfectly stacking helices. Similarly, the BB interaction is monitored by the FRET change between the FAM$_3$-B/TAMRA$_3$-B dyes, located respectively at the helices 14 and 19 of the B tip (c). Note that isologous interactions result from stacking of two monomers at the same interface type. This is achieved upon face-to-face orientation of the two units and further 60° rotation of one monomer around its central axis. For this reason, placement of a FAM and a TAMRA reporter strand at distant sites of a monomer tip leads to two pairs of FRET sensors. Finally, simultaneous detection of all types of interactions, i.e. AB, AA and BB, as in the random stacked oligomer, is achieved by using both the FAM$_2$-A/TAMRA$_2$-A pair and the FAM$_3$-B/TAMRA$_3$-B pairs (d). Each patch is indicated in color-code (A in blue and B in orange, with increasingly darker colors indicating a higher extent of helical protrusions).
Figure S3. Analysis of dimer association by isothermal FRET assays. The initial rate of monomer stacking was measured during the first linear phase of the reaction (ca. 4 min) at 30°C and plotted as a function of the initial monomer concentration. The slope of the linear fit indicates the initial association rate coefficient ($k_a$; Eq. 8 in Suppl. Note 1). Values for stacking at the AA (a) and BB (b) interfaces are, respectively, $(14.4 \pm 0.1) \times 10^{-2}$ min$^{-1}$ and $(30 \pm 4) \times 10^{-2}$ min$^{-1}$, indicating that self-association at the B tip is kinetically favored.
Figure S4. Time-course AGE characterization of dimer formation. Activation strands for stacking at the AA (a) or BB (b) interface were added to a pre-assembled inactive monomer and the products of the reaction, either at 25 °C or 40 °C (bottom and upper panels, respectively) were characterized by agarose gel electrophoresis by freezing an aliquot of the mixture in liquid nitrogen at different time points (from 0 to 20 hours). Successful formation of the dimer is visible by the appearance of a slow-migrating band and concomitant disappearance of the initial monomer band (lane AB). Samples at 0 min were taken directly after mixing monomers and stacking strands. In all cases, dimerization occurs rapidly within the first minutes of the reaction, although a slightly higher rate can be discerned for BB stacking, particularly at 25°C (b, lower panel). A clearer differentiation between the two assembly rates was obtained by FRET and AFM analysis (see main manuscript and Figure S5). Gel running conditions: 1% agarose in 1X TBEMg buffer at 4°C for 2:45 hr; ethidium bromide staining. Lane named AB contains a pre-assembled inactive monomer as reference.
**Figure S5. Time-course AFM characterization of dimer formation.** Activation strands for stacking at the AA or BB interface (light and dark blue dots, respectively) were added to a pre-assembled inactive monomer and the products of the reaction, either at 25 °C (a) or 40 °C (b) were characterized by manual counting of the structures imaged by atomic force microscopy at different time points (from 0 to 120 min). Each data point shown in the graph was taken from over 400 structures. Initial monomer concentration was 20 nM, final dimer concentration is expected to be 10 nM. Samples were collected as described in Figure S4 and diluted to 2 nM in 1X TEMg16 prior to AFM imaging. The experimental points were fitted with an irreversible second-order reaction kinetics, with rate coefficient $k_a^*$ for the association of the monomer into a dimer (Eq. 7 in Suppl. Note 1). Fit was executed using a built-in MATLAB script. Note that the approximation to a second-order kinetics leads to similar conclusions as those reported by analysis of the initial rates in time-dependent FRET experiments (Figure S3 and Suppl. Note 1), with BB stacking being much faster than AA stacking at 25°C. The association rate at 40°C is probably too fast to allow reliable data collection in the initial phase of the reaction, leading to counteractive results.
Figure S6. Agarose gel characterization of one-step and two-step dimer assembly. Stacked dimers were self-assembled according to either a one-step (lanes indicated as 1) or two-step (lanes 2) procedure, leading to equilibrium products with a similar electrophoretic mobility on an agarose gel. In the one-pot assembly, stacking staples for the AA or BB interface were added to the mixture of oligonucleotides needed for formation of the inactive monomer and the whole ensemble of DNA strands was subject to thermal annealing, as described below (Suppl. Materials, section 6). In the two-step assembly procedure, stacking strands were added at constant temperature (40 °C for 20 h) to a pre-assembled inactive monomer obtained using the same thermal gradient as in the one-pot assembly. All structures in the gel were obtained from a 50 nM monomer core using a tenfold excess of AA or BB stacking staples. Gel running conditions: 1% agarose in 1X TBEMg buffer at 4 °C for 2:45 hr; ethidium bromide staining. Lane named AB contains a pre-assembled inactive monomer as reference.
Figure S7. Time-course characterization of one-step dimer assembly. A 50 nM monomer assembly mixture was treated with a 10-fold excess of AA (a) or BB (b) stacking strands and exposed to a thermal gradient from 75 °C to 40 °C at -0.1 °C/min. Samples were collected every 5 °C and the assembly reaction was stopped by freezing it in liquid nitrogen. Several bands of intermediate monomeric structures as well as non-migrating aggregates were observed at temperatures higher than 55 °C, after which stacking of isologous interfaces initiates (lane highlighted in yellow). Interestingly, whereas for the BAAB dimer the transition from 55°C to lower temperatures reflects into an increased migration speed of the final product (a, cfr. lane 55 with lanes 50 to 40), the opposite is true for the ABBA dimer, with a slight decrease of the electrophoretic mobility of the structure (b, cfr. lane 55 with lanes 50 to 40). In both cases, the final equilibrium structures correspond to the expected origami dimers, as visible by AFM imaging of one-step dimerization reactions using stacking strands for the AA (c) or BB (d) interface. Imaging was performed in air, using a 2 nM sample solution diluted in TEMg16, as described in the Suppl. Material (section 5, with 640 samples/line). Both reactions yielded mostly dimeric structures with monomers likely resulting from bond breakage upon contact with the mica surface.
Figure S8. Analysis of the first-derivative thermal profiles of dimers. The cooling (blue lines) and melting profiles (orange lines) of the BAAB (dark color) and ABBA (light color) dimers were plotted as first-derivative vs temperature, to individuate the temperature at the turning point of the transition ($T_m$ in Table 1 of the main manuscript).
Figure S9. Concentration-dependent FRET profiles of dimer melting. Pre-assembled BAAB (a) and ABBA (b) dimers at 41, 33 and 25 nM (blue, orange, and grey curves, respectively) were heated from 25°C to 75 °C with a scan rate of 0.1 °C/min. The unfolding of the structure was monitored over time as the change in the FRET efficiency of the FAM/TAMRA labeled construct (in reference to a FAM-only analog). The data show that, for both dimers, the concentration of the sample (i.e. the concentration of the scaffold initially present in solution) does not appreciably affect the melting temperature and the profile of the thermal curves.
Figure S10. Temperature-dependent FRET profiles of various BAAB dimer interface constructs. BAAB dimers were assembled according to a one-pot procedure, starting from a mixture of scaffold and core strands in presence of 10-fold excess stacking strands for the AA interface. The assembly (blue curve) and disassembly (orange curve) processes were monitored by FRET at -/+0.1 °C/min, applying the labelling strategy depicted in Figure S2 (the position of the dyes is indicated by magenta and green circles). Stacking strands were added to induce the formation of blunt-end duplexes with unpaired portions of the scaffold. We designed the AA interface such to have an increasing number of blunt-end duplexes facing each other, with stacked (0-bp gap)/1-bp gapped helices varying from 16/0 (a) to 16/8 (e). Blunt-end duplexes that face each other at the opposite sides of adjacent monomer tips may effectively stack, i.e. they build a continuous duplex with no helical gap (dark blue circles), or can be separated by 1-bp gap (light blue circles). Blunt-end duplexes that lack their corresponding partner on the opposite side of the interface (i.e. they are unmatched) are indicated by a cross. Unbuilt duplexes are in white. Interestingly, hysteresis keeps to a minimum until the full construct 16/8 is built (e).
Figure S11. Temperature-dependent FRET profiles of various ABBA dimer interface constructs. ABBA dimers were assembled according to a one-pot procedure, starting from a mixture of scaffold and core strands in presence of 10-fold excess stacking strands for the BB interface. The assembly (blue curve) and disassembly (orange curve) processes were monitored by FRET at -/+0.1 °C/min, applying the labelling strategy depicted in Figure S2 (the position of the dyes is indicated by magenta and green circles). Stacking strands were added to induce the formation of blunt-end duplexes with unpaired portions of the scaffold. We designed the BB interface to have an increasing number of blunt-end duplexes facing each other, with stacked (0-bp gap)/1-bp gapped helices varying from 8/0 (a) to 8/16 (j). Blunt-end duplexes that face each other at the opposite sides of adjacent monomer tips may effectively stack, i.e. they build a continuous duplex with no helical gap (dark orange circles), or can be separated by 1-bp gap (light orange circles). Blunt-end duplexes that lack their corresponding partner on the opposite side of the interface (i.e. they are unmatched) are indicated by a cross. Unbuilt duplexes are in white. Interestingly, above 10 unstacked blunt helices, the cooling and melting profiles do not overlap and thermal hysteresis appears (g-j).
Figure S12. Temperature-dependent FRET profiles of various stacked dimer versions. The cooling (a and c) and melting (b and d) profiles of Figure S10-S11 are here represented as a function of the number of stacked over 1-bp gapped helices (the two numbers are separated by a slash “/”) for both the BAAB (a and b) and the ABBA (c and d) dimers. It is evident that the thermal behavior of the ABBA dimer is strongly affected by the number of helical patches at the isologous interface, with formation of unstacked pairs of 16 helices (from 8/0 to 8/16) spanning a change in cooling or melting temperature of more than 20 °C. Comparison between the AA and the BB interface can be done by observing the change in the thermal profiles resulting from adding the last 8 helices, which are separated by 1-bp gap. Specifically, from construct 16/0 to 16/8 for the BAAB dimer (a and b) and from construct 8/8 to 8/16 for the ABBA dimer (c and d). In both cases, the total number of blunt helices varies from 16 to 24 and hysteresis is generated by the formation of gapped duplexes at the isologous interface. This is due to a shift in the melting profile that is larger than its corresponding shift in the cooling profile. However, this effect is much more evident for the ABBA rather than the BAAB dimer. Altogether, these data suggest that the formation of blunt-end helices at the isologous interface of stacked monomers effectively contributes to the thermal hysteresis of the dimer in a sequence-dependent fashion. We presume that above a certain threshold, structural distortions may indeed occur that favor the interaction between opposed blunt ends, despite these latter are virtually not designed to stack properly.
Figure S13. Thermal features of different stacked dimer versions. The cooling and melting temperatures (blue and orange bars, respectively) and the theoretical energies of base stacking (indicated by a yellow line) are reported for each partially stacked dimeric construct, from 16/0 to 16/8 (BAAB dimer in a) and from 8/0 to 8/16 (ABBA dimer in b). The melting temperature ($T_m$) is defined as the temperature for which $\frac{d^2\theta}{dT^2} = 0$ (left axis). The theoretical energy of base stacking (right axis) was calculated considering full stacking of all formed helices, including both those that effectively stack and those which are not supposed to stack properly, due to the 1 bp-gap in between. When comparing the formation of the last 8 duplexes, one can note that the difference between the melting and cooling temperature is larger for the ABBA dimer when compared to the BAAB dimer, indicating a large hysteresis extent at the BB isologous interface.
Figure S14. Isothermal time-dependent FRET measurement of \((AB)_n\) filament formation.
The head-to-tail association of the AB monomer into the ordered \((AB)_n\) oligomer was triggered by addition of activation strands that induce the hybridization of the A tip of one monomer with the B tip of an adjacent monomer. The extent of reacted AB monomer is obtained through normalization of the FRET efficiency and transformed into concentration values assuming that, at the equilibrium, all monomers have reacted and are converted into oligomers. The plateau of each FRET curve is therefore set to the initial monomer concentration. This assumption is validated by the almost negligible values of critical monomer concentration observed by AFM (Figure S23) and AGE (Figure S24). Finally, the rate of filament growth was analyzed for increasing initial concentrations of inactive monomer (indicated by red to purple curves) and at three different temperatures (30°C, 35°C and 40°C; a, b and c, respectively). The initial rate of filament formation increases with the initial monomer concentration in an approximatively linear fashion, enabling to calculate the initial association rate coefficient by the slope of the linear fit for each temperature value (d to f). Finally, the value of activation energy for the reaction under study is derived by the slope of the Arrhenius plot applied to three different temperature values (g).
Same data treatment applies to Figures S15-S20.
Figure S15. Isothermal time-dependent FRET measurement of rand_all filament formation. The “random” stacking association of the AB monomer into a linear oligomer of unpredictable (and undefined) periodicity was triggered by the addition of activation strands that induce both the heterologous (AB) and the isologous (AA and BB) stacking of monomer tips. The rate of filament growth was analyzed as described in the legend to Figure S14.
Figure S16. Isothermal time-dependent FRET measurement of rand_AB filament formation. The heterologous association of the monomer at the AB interface was monitored over time in the context of a randomly stacked filament (rand_all) that contains all three modes of stacking associations (AB, AA and BB), with AB being the only interface labeled. The rate of filament growth was analyzed as described in the legend to Figure S14. In this case, concentration values were estimated according to the fraction of rand_AB oligomers observed by AuNP labelling of random filament at equilibrium (Figure S21).
Figure S17. Isothermal time-dependent FRET measurement of rand_AA filament formation. The isologous association of the monomer at the AA interface was monitored over time in the context of a randomly stacked filament (rand_all) that contains all three modes of stacking associations (AB, AA and BB), with AA being the only interface labeled. The rate of filament growth was analyzed as described in the legend to Figure S14.
**Figure S18. Isothermal time-dependent FRET measurement of rand_BB filament formation.** The isologous association of the monomer at the BB interface was monitored over time in the context of a randomly stacked filament (rand_all) that contains all three modes of stacking associations (AB, AA and BB), with BB being the only interface labeled. The rate of filament growth was analyzed as described in the legend to Figure S14.
Figure S19. Isothermal time-dependent FRET measurement of (ABBA)$_n$ filament formation. The isologous association of the ABBA dimer at the AA interface was monitored over time upon addition of AA activation strands. The rate of filament growth was analyzed as described in the legend to Figure S14.
Figure S20. Isothermal time-dependent FRET measurement of (BAAB)$_n$ filament formation. The isologous association of the BAAB dimer at the BB interface was monitored over time upon addition of BB activation strands. The rate of filament growth was analyzed as described in the legend to Figure S14. Interestingly, we observed a rapid increase in the FRET signal followed by a monotonic decrease after only 30 min, particularly at high monomer concentrations (green curve). We presume that the limited shape-complementarity of the DNA origami units at the B interface enhances the rate of B-to-B association at the expenses of a less stable bond, eventually preventing the formation of long polymers. This effect appears to be more evident for the association of dimers into polymers, when compared to the association of monomers into dimers.
Figure S21. Isothermal time-dependent FRET measurement of (AB)$_n$ filament formation with varying number and positioning of hybridization sites. The head-to-tail association of the AB monomer into the ordered (AB)$_n$ oligomer was triggered by addition of activation strands that induce the full or partial hybridization of the A tip of one monomer with the B tip of an adjacent monomer. Partially hybridized constructs derive from the activation of a limited number of helices (from 8 to 14) at distinct relative positions of the interface (inner, outer, left or right). Filaments were obtained by mixing 50 nM inactive (and fluorescently labelled) monomer with ten-fold excess of hybridization strands in TEMg16 (same procedure as for the other FRET curves). Monomers were diluted to 15 nM and MgCl$_2$ concentration was adjusted to 20 mM. The FAM emission was recorded over 24h at 40°C and triplicates were measured per donor and donor/acceptor sample. Hybridization at the outer helices occurs very rapidly (12 pM/s), while hybridization at the inner helices is much slower (ca. 5 pM/s). We presume that the outer helices, being exposed to the solution, are more easily accessible to the fuel strands and can rapidly hybridize. Conversely, the inner helices are partially buried by unpaired scaffold segments from the surrounding regions of the structure and may be more difficult to reach. The predominant role of the accessibility of the binding sites is confirmed by the fact that hybridization of the full structure, which occurs at 22 out of 24 helices (green curve and bar), is faster than the formation of the inner-bound polymer (8/22, purple curve and bar), as indeed expected, but slower than the formation of the outer-bound polymer (14/22; blue curve and bar), despite involving more binding interactions. The observed difference between the right (14/22) and the left (12/22) constructs can be explained by the distinct number of helices involved, as well as by possible contributions from the specific base sequences and most probably to the structural local environment nearby the FRET pair.
Figure S22. Statistical distribution of random stacking populations. Upon formation of the randomly stacked filament, the A subunit of each AB monomer was topographically marked with two gold nanoparticles (inset in a) as described in the Suppl. Material (section 3). A 2 nM solution of inactive AB monomer was treated with an excess of stacking strands for random polymer assembly at 40 °C for 20 h. The relative populations of the three possible stacking interactions (AB, AA and BB) were estimated from manual counting of about 900 stacked interfaces, collected from more than 80 TEM images (exemplary image in a). The results (b) indicate that stacking at the AB interface occurred in 49% of possible associations, whereas stacking at the AA or BB interface occurred with a frequency, respectively, of 28 and 23% ($n = 892$). Standard deviations refer to the deviations among all images analyzed. The relative fraction of stacking interactions observed in the random filament nicely matches with the number of properly stacked blunt-end helices required for formation of the monomer-to-monomer association (i.e. AB > AA > BB, corresponding to 24 > 16 > 8 stacked helices). This point is further discussed in Suppl. Note 3.
Figure S23. Time-course monitoring of filament formation by AGE. Activation strands for hybridization (a) or random stacking at both the A and B tips (b) were added to a pre-assembled inactive monomer at 40 °C for 20 h or 22 h. Samples at 0 min were taken directly after addition of polymerization staples and the reaction was stopped at increasing time points through immersion of the solution sample in liquid nitrogen. Successful polymerization can be inferred by the gradual disappearance of the initial monomer band (lane AB) and concomitant increase in the intensity of the signal in the gel pockets (due to formation of non-migrating aggregates). Hybridization samples show distinct monomer, dimer and multimer bands up to 2 hours after onset of the polymerization reaction with a progressive decrease of band intensity. Random stacking showed instead a faster kinetic behavior with only weak and indistinguishable bands of the 0 min sample taken directly after staple addition. Gel running conditions: 1% agarose in 1X TBEMg buffer at 4°C for 3 hr; ethidium bromide staining. Lane named AB contains a pre-assembled inactive monomer as reference.
The concentration of the monomer at the equilibrium was estimated by AFM, counting the average number of monomeric structures present in a solution of polymer after treating the inactive AB monomer with an excess of activation strands at a given temperature (30°C, 35°C or 40°C) and incubating the reaction up to 16 h. A 1 nM solution (10 µl) of monomer in TEMg20 was used as reference and identical volumes and scan sizes (10 µm x 10 µm) were used in all samples to enable their reliable quantitative comparison. About 10 images were acquired for each sample at the equilibrium. Samples analyzed were the (AB)$_n$ and the (AB)$_{rand}$ filaments (black and green bars). As the data show, although both polymerization reactions consume almost the entire amount of initially available monomer, the hybridization-driven polymerization rests to a defined and non-negligible amount of building unit that decreases with increasing temperature, whereas the stacking-triggered polymerization appears to be essentially complete and temperature-independent. These data can be better understood in view of our FRET data (main manuscript, Figure 4). Indeed, we observed that whereas the hybridization-driven assembly of the DNA origami monomer mostly proceeds through tip elongation or formation of newly formed short oligomers, stacking-driven assembly preferentially occurs through fragmentation and condensation events, thus essentially consuming almost all monomer available.
Figure S25. Proposed model of monomer association. Formation of (AB)$_n$ filaments by base hybridization of activated monomers requires the displacement of (half of the) strands from the scaffolds of connecting interfaces (left panel). Conversely, stacking of activated monomers may proceed without intermediate reaction steps (right panel).
Figure S26. Concentration-dependent FRET profiles of filament melting. Pre-assembled filaments, formed by head-to-tail monomer hybridization (a) or by random stacking of monomers (b), were heated from 20°C to 75 °C with a scan rate of 0.1 °C/min. Initial monomer concentrations were 50, 40 or 30 nM (blue, orange and grey curves, respectively) The unfolding of the structure was monitored over time as the change in the FRET efficiency of the FAM/TAMRA labeled construct (in reference to a FAM-only analog). The data show that, in both filaments, the concentration of the sample (i.e. the concentration of the scaffold initially present in solution) does not appreciably affect the melting temperature and the profile of the thermal curves.
Figure S27. Melting FRET profiles of DNA origami filaments. Once assembled, fluorescently labelled DNA origami filament structures were melted from 25°C to 75°C at 0.1°C/min and the FRET efficiency change was recorded over time to estimate the extent of folded fraction. The folded fraction ($\theta$) was plotted versus temperature (a) to determine the melting temperature at $\theta = 0.5$ ($T_{0.5}$) and at $d^2\theta/dT^2 = 0$ ($T_m$). The plots of $\theta$ vs. $T/T_{0.5}$ (b) better illustrate the differences in the melting behavior of the filaments (enlarged view is shown in the inset to b). Stacking polymers were assembled randomly from monomers, whereas the periodic (AB)$_n$ was obtained upon addition of hybridization strands to an inactive AB monomer. All filaments were obtained as described in the Suppl. Materials (section 6). The corresponding hybridization or stacking bonds were labelled with FRET pairs as shown in Figure S2. The hybridized filaments show the highest thermal stability ($T_{0.5} = 63.3°C$), with a melting temperature that is about 2 °C higher than that of all other stacked polymers. This result well matches with our AFM and FRET data (Figure 4 of the main manuscript), which demonstrate that the hybridization forces that keep together the monomeric units, despite being kinetically disfavored, may result into structurally more stable polymer chains. Interestingly, stacking at the AA interface shows the steepest melting profile, indicating a rapid disassembly of the structure around the melting temperature.

| heating   | $T_{0.5}$ | $T_m$ |
|-----------|-----------|-------|
| (AB)      | 63.3      | 69.0  |
| rand_all  | 66.9      | 62.3  |
| rand_AB   | 66.9      | 62.5  |
| rand_AA   | 61.0      | 61.1  |
| rand_BB   | 60.7      | 62.6  |
Supplementary Materials and Methods

1. Materials and chemicals
Oligonucleotides were purchased from Sigma-Aldrich. Unmodified oligonucleotides were delivered lyophilized and desalted in 96-well plates. 6-carboxyfluorescein (6-FAM) or carboxytetramethylrhodamine (TAMRA) modified oligonucleotides were delivered in HPLC-purified form. The single-stranded M13mp18 scaffold was produced from phage DNA (Affymetrix) in *E.coli* XL1-Blue competent cells (Agilent technologies), as previously described.1 1x TEMg16 (5 mM Tris base, 1 mM EDTA, 16 mM MgCl2, pH 8), 1x TEMg20 (5 mM Tris base, 1 mM EDTA, 20 mM MgCl2, pH 8), as well as 1x TBEMg (40 mM Tris base, 40 mM boric acid, 2 mM EDTA, 12.5 mM MgCl2, pH 8) buffers were used.

2. Design and assembly of DNA filaments
DNA origami structures were designed with caDNAno (www.cadnano.org v2.2.0), as previously reported,2 and assembled using a 1:10 molar ratio between the M13mp18 scaffold (50 nM) and each of the staple strands in 1x TEMg16 buffer. Thermal annealing was performed on a Thermocycler Mastercycler nexus gradient (Eppendorf) according to the following program: 65°C for 5 min, 65-55 °C (-1°C/5 min), 55-30°C (-1°C/15 min), 30-20°C (-1°C/min) and hold at 20°C. Upon adjustment of the Mg2+ concentration to 20 mM, multimers were assembled by mixing intermediate species with initiator staple strands in 10-fold excess for 20 h at 40 °C (if not stated differently). DNA origami structures were either directly used for experiments or purified using 100 kDa MW cutoff filtration devices (Amicon Ultracel), prior to their conjugation to gold-nanoparticles (see below). A complete list of sequences is provided at the end of this document.

3. AuNP-labelling of DNA origami
Gold nanoparticles (AuNP) with a diameter of 20 nm were coated with 1000-fold excess of a thiol-modified oligonucleotide (ThiC6-TAATAATAATAATAAT) according to published procedures.3-4 The oligo linked to the AuNP is complementary to the sequence of eight protruding arms, positioned on top of the A subunit of the monomer structure. Upon thiol deprotection using tris(2-carboxyethyl)phosphine (TCEP), the reaction mixture was desalted by size exclusion chromatography (NAP DNA Purification Columns, GE) and finally concentrated on 3 kDa MW cutoff filtration devices (Amicon Ultra 0.5 mL, Millipore). AuNPs were washed with 1 % Tween 20 and resuspended in 0.05 % Tween 20 and eventually conjugated with the freshly-deprotected thiol-modified oligonucleotide. Conjugation was performed by stepwise addition of NaCl until a concentration of 200 mM and the final product was washed three times with ddH2O. A 2 nM solution of purified AB monomer, decorated with eight protruding arms on top of the A subunit, was treated with initiator stacking strands at
40 °C for 20 h to promote formation of randomly stacked filaments (AB\textsubscript{rand}). After polymerization, the AuNPs coated with the complementary oligonucleotide sequences were added in a 1:1 ratio and incubated at room temperature for four days. The samples were finally analyzed by negative stain TEM.

4. Negative stain transmission electron microscopy
Samples for TEM were prepared as previously described.\textsuperscript{2, 5} Briefly, DNA filaments were absorbed onto freshly glow-discharged copper grids (covered by a 6 nm continuous carbon film) and stained twice with 1% uranyl formate. The grids were blotted dry, and imaging was done on a Zeiss EM 910 transmission electron microscope with 120 kV accelerating voltage and a LaB6 cathode. Alternatively, a JEOL JEM 1400 Plus with a LaB6 Filament and a TVIPS TemCam-F416 camera was used for TEM imaging.

5. Atomic force microscopy
A 500 µl solution of AB monomer (3 nM) or dimer sample (2 nM) was prepared in TEMg20 buffer containing 50 mM NaCl. The sample was deposited on a freshly cleaved mica surface (Plano GmbH) and adsorbed for 3 min at room temperature. 4 µm x 4 µm areas were scanned with 400 samples/line at 6.25 Hz in Fast Tapping Mode resulting in the recording of one image every 32 seconds using a BioScope Resolve microscope (Bruker) equipped with a Nanoscope V controller. Super sharp probes with a 1 nm tip and 0.25 N/m spring constant (FASTSCAN\_D\_SS tips, Bruker) were used for scanning. The drive amplitude was typically set at 200 mV and the amplitude setpoint was adjusted between 600 and 900 mV. After successful imaging of the individual building units, 500 µl of initiator strands (100 nM in TEMg20 buffer containing 50 mM NaCl) were added in situ to promote the polymerization reaction. Alternatively, polymerization equilibrium samples were imaged in air after depositing 10 µl of a 1 nM solution on a freshly cleaved mica surface and adsorbing for 3 min before washing with ddH\textsubscript{2}O and subsequent drying. Samples were scanned using the ScanAsyst mode with ScanAsyst Air tips (Bruker). All measurements were conducted at room temperature and images were analyzed using the NanoScope Analysis v2.0 software.

6. Temperature-dependent FRET spectroscopy
Stacked dimers were prepared by cooling down a mixture of scaffold, core staples and stacking strands from 75 °C to 25 °C at a rate of -0.1 °C/min. Upon assembly, the structures were melted at +0.1 °C/min over the same temperature range. Filament structures were instead polymerized from pre-assembled monomers upon incubation in TEMg20 for 20 h at 40 °C. The so-obtained equilibrium structures were then melted from 25 °C to 75 °C with a rate of +0.1 °C/min. For each structure, two samples were prepared: one sample contained the donor-
labelled strand and the oligonucleotide corresponding to the acceptor position, the other sample contained the donor- and acceptor-labelled strands. During the cooling and heating process, the fluorescence signal intensity of the donor (exc. 450-490 nm/em. 510-530 nm) was collected in both the donor-only \( (I_D) \) and donor-acceptor \( (I_{DA}) \) sample and the two intensities were used to calculate the FRET efficiency as \( E = 1 - \frac{I_{DA}}{I_D} \). The melting temperature \( (T_m) \) was determined as the maximum of the first derivative of the normalized FRET efficiency thermal curve, whereas the temperature corresponding to \( E = 0.5 \) was indicated as \( T_{0.5} \).

7. Isothermal FRET spectroscopy

Isothermal FRET spectroscopy experiments were performed for both dimers and all possible polymerization modes at 30, 35 and 40 °C and different initial concentrations of monomers or pre-assembled dimers (from 5 nM to 40 nM). Kinetic FRET curves were recorded on a Tecan Spark 10M in clear-bottom 96 well microplates with black walls (Greiner) covered with a clear adhesive foil. A two-fold excess of fluorescently labeled oligonucleotides was added to the core assembly mix and the corresponding unlabeled oligo was used in the donor-only control (Fig. S2). 6- FAM was used as the donor dye, whereas TAMRA was used as the acceptor fluorophore. Magnesium chloride was added to the assembly mixture to reach a concentration of 20 mM and triplicates of 100 µl were thermally equilibrated for at least 15 minutes before starting the measurement. After recording a stable baseline for the unreacted building blocks, a ten-fold excess of polymerization-inducing strands was added to the reaction mixtures using a multichannel pipette and the emission fluorescence intensity of the 6-FAM dye was measured over time until equilibrium was reached. Measurements were performed from the bottom of the plate after 3s shaking, 1s settling and with 15 flashes. The gain was adjusted manually to match the fluorescence intensity of each sample. Time offset due to staple addition was determined to be 30 sec. Thus, the first data point of polymerization was set to 30s with following time points adjusted accordingly.

Equilibrium perturbation of filaments

Fluorescently labeled monomers were prepared from 50 nM scaffold and 47.5 nM fluorophore labeled oligonucleotides. Subsequently, the solution was diluted to a final monomer concentration of 20 nM in TEMg20 and polymerization via hybridization or random stacking was initiated upon addition of 10-fold excess of staples. Triplicates were incubated at 40 °C and the FAM-emission intensity was recorded over time. When the polymerization equilibrium was reached, an equimolar amount of freshly prepared unlabeled monomer was added to the solution of filaments and the change in the FAM-emission intensity was recorded over time to observe polymer turnover off-rate.
Experimental data were saved in .xlsx format as time-resolved relative fluorescent units (RFU). A custom python script was developed to perform data analysis, enabling to import reaction temperature, concentration, gain, recorded wells, reaction type, measurement time points and RFU data of the triplicates. The script allowed to select the best linear fit with an appropriate set of experimental data points. Imported data were then used to calculate the FRET efficiency according to Eq. 1 applied to the average value of each triplicate (at a given concentration and temperature):

\[ E = 1 - \frac{I_{DA}}{I_D} \]  

Eq. 1

with \( I_{DA} \) and \( I_D \) being, respectively, the emission fluorescence intensities of the fluorescein dye in the donor-acceptor and donor-only sample. The standard error of the FRET efficiency (\( \sigma_{FRET} \)) was calculated as given in Eq. 2:

\[ \sigma_{FRET} = \sqrt{\left(\frac{1}{\bar{x}_D}\right)^2 \times \sigma_{DA}^2 + \left(\frac{\bar{x}_{DA}}{\bar{x}_D}\right)^2 \times \sigma_D^2} \]  

Eq. 2

where \( \bar{x}_{DA} \) and \( \bar{x}_D \) were, respectively, the mean value of the FRET efficiency for the donor-acceptor and donor-only sample, with \( \sigma_{DA} \) and \( \sigma_D \) being the corresponding standard deviations. The extent of FRET efficiency was assumed to be proportional to the extent of reacted monomer (or pre-assembled dimer), with the maximal FRET value at the equilibrium scaled to the original concentration of the reacting unit (\( c_0 \)). This procedure is justified by the fact that at the equilibrium, \( c_{crit} << c_0 \), that is, the critical concentration of the monomer or the reacting unit is negligible with respect to the initial total concentration and almost all available building blocks have reacted (Figure S23).

**8. Theoretical models and simulations**

All simulations and fits of the experimental curves according to the ODE functions reported in this work have been done using a MATLAB home-made script that applies the ode45 code to solve the ODE and the lsqcurvefit solver to find the best rate coefficients that minimize the difference between the experimental curve and the numerical solution of the ODE.
Supplementary Notes

1. Kinetic analysis of isothermal FRET profiles

Activation of the AB monomer and its self-association into one of the two possible dimers ABBA or BAAB (D) has been modeled as a two-step reaction, as described below:

\[ \begin{align*}
    K_{\text{act}} & \quad AB + aS \rightleftharpoons AB^* \\
    2AB^* & \xrightleftharpoons[k_d]{k_a} D
\end{align*} \]

**Scheme 1. Proposed mechanism of monomer activation and dimerization.**

Here we assume that, upon addition of activation strands (aS), the inactive monomer (AB) is activated at its terminal edges into the AB* form capable to self-associate into the dimer D. We assume that the first (pre-equilibrium) step is characterized by the equilibrium association constant \( K_{\text{act}} \), described by Eq. 3. Please note that here and in the following equations, the square brackets typically used to indicate the concentration of a species are omitted, for simplicity.

\[ K_{\text{act}} = \frac{[AB^*]}{[AB][aS]} \quad \text{Eq. 3} \]

\[ AB^* = K_{\text{act}} \cdot (AB) \cdot (aS) \quad \text{Eq. 4} \]

The second step of the reaction describes the binding of two monomers into a dimer with an association rate coefficient \( (k_a) \) and a dissociation rate coefficient \( (k_d) \). The rate equation for the dimerization process can be thus expressed by the ordinary differential equation (ODE) given below (Eq. 5):

\[ \frac{dD}{dt} = k_a(AB^*)^2 - k_dD = k_a(K_{\text{act}})^2(AB)^2(aS)^2 - k_dD \approx k_a'(AB)^2 - k_dD \quad \text{Eq. 5} \]

As the activation strands are typically used in large excess in respect to the monomer, their concentration can be considered approximatively constant, at least at the beginning of the reaction. This allows to describe the process as a pseudo second-order reaction with respect
to the AB concentration, characterized by an apparent association rate coefficient ($k_a'$). Given
the initial monomer concentration ($c_0$) and the law of mass conservation at any time, $t$:

$$AB = c_0 - 2D$$  \hspace{1cm} \text{Eq. 6}

Eq. 5 can be rewritten as

$$\frac{dn}{dt} \approx k'_a (c_0 - 2D)^2 - k_a D$$  \hspace{1cm} \text{Eq. 7}

In our hands, each single experimental curve could be well fitted with Eq.7; however, a global
fit of all curves was not satisfactory, suggesting that the model might be unsuitable to describe
the mechanism of the reaction or that the observed FRET changes, which indeed report only
that part of the process around the monomer interfaces, might possibly overlook some other
important reaction steps. We therefore decided to describe the dimerization of the monomer
and all our isothermal FRET curves by the initial rate of the reaction. This assumption is
justified by the fact that the first phase of the reaction is linear in all constructs and
concentrations analyzed and thus offers a reliable way to compare different scenarios on the
same rationale. Accordingly, we describe all FRET time-dependent profiles by the apparent
association rate coefficient ($k_a^{\text{in}}$) in the initial phase of the reaction, as reported in Eq. 8:

$$\left. \frac{dD}{dt} \right|_{t=0} \approx k_a^{\text{in}} c_0$$  \hspace{1cm} \text{Eq. 8}

The initial linear slope was determined based on a comparison between $R^2$ values for up to
120 data points, including a minimum of five data points. This operation was performed
automatically with the aid of a script written in python.
2. Analysis of the thermal hysteresis profiles

The thermal hysteresis observed during the assembly and disassembly of the DNA origami dimers was analyzed as previously reported for other DNA structures. Our data indicate that the process is independent on the concentration of the scaffold (Figure S9). Melting temperatures for both processes and both ABBA and BAAB dimers vary by no more than 1°C for concentrations between 25 nM and 40 nM. This enables in principle to simplify the process to a quasi-intramolecular transformation. Despite the folding of a DNA origami is surely too complicated to be described by such a simple model, the melting of the structure should allow for such an approximation. Indeed, our FRET curves are not indicative of the global transformation of the DNA origami structure, rather they report exclusively the structural changes in the close vicinity of the fluorophore dyes, at the interface of two stacked monomers. We thus restricted our thermal analysis to the microdomain localized nearby the FRET pair(s) and applied the pseudo-equilibrium model described in detail below to simulate and compare the thermal behaviors of the dimers at theoretical equilibrium conditions. Given this premise, the association/dissociation of the origami microdomain can be described as follows:

\[
\frac{k_{ON}}{U} \rightleftharpoons \frac{k_{OFF}}{F}
\]  

Eq. 9

with U and F being, respectively, the unfolded and folded state of the structure. At any time, the following expression keeps valid: \(C_0 = U + F\), with \(C_0\) being the initial and total concentration of the dimeric structure. We now identify \(\theta\) as the folded fraction, \(\theta = F/C_0\). Thus \(U = 1-\theta\). The rate of formation of the folded structure can be therefore described as:

\[
\frac{d\theta}{dt} = \frac{k_{ON}U}{C_0} - \frac{k_{OFF}F}{C_0} = k_{ON} (1 - \theta) - k_{OFF}\theta
\]  

Eq. 10

The curve that describes the progress of the reaction over time can be transformed into a temperature profile, once the scan rate is given:

\[
\frac{d\theta}{dt} = \frac{d\theta}{d\tau} \times \frac{d\tau}{dt}
\]  

Eq. 12

In our case, \(d\tau/dt = \pm 0.1^\circ C/\text{min}\), for the heating (dissociation) and cooling (association) process, respectively. In presence of hysteresis, the cooling and heating profiles do not
overlap. For each process, however, Eq. 11 is valid, leading to a system of two equations in two variables ($k_{ON}$ and $k_{OFF}$) that must be satisfied simultaneously:

\[
\begin{align*}
\frac{d\theta_c}{dt} &= k_{ON}(1 - \theta_c) - k_{OFF}\theta_c \\
\frac{d\theta_h}{dt} &= k_{ON}(1 - \theta_h) - k_{OFF}\theta_h
\end{align*}
\]  

Eq. 13

Solving this system of equations leads to the values of $k_{ON}$ and $k_{OFF}$, reported below:

\[
k_{ON} = \frac{h\theta_c-c\theta_h}{(\theta_c-\theta_h)} \quad \text{Eq. 14}
\]

\[
k_{OFF} = \frac{h(1-\theta_c)-c(1-\theta_h)}{(\theta_c-\theta_h)} \quad \text{Eq. 15}
\]

with

\[
h = \frac{d\theta_h}{dt} \times \frac{dt}{d\theta_h} = \frac{d\theta_h}{d\theta_c}
\]  

Eq. 16

\[
c = \frac{d\theta_c}{dt} \times \frac{dt}{d\theta_c} = \frac{d\theta_c}{d\theta_h}
\]  

Eq. 17

As evident from Eq. 14 and Eq. 15, the rate coefficients ($k_{ON}$ and $k_{OFF}$) obtained from the experimental curves will be temperature dependent. The next step is to apply the Arrhenius equation to both the association (ON) and dissociation (OFF) reaction.

\[
k_{ON} = A_{ON} \times e^{-\frac{E_{ON}}{RT}} \quad \text{Eq. 18a}
\]

\[
k_{ON} = \frac{k_{ref}^{ON}}{e^{-\frac{E_{ON}}{RT_{ref}}}} \times e^{-\frac{E_{ON}}{RT}} \quad \text{Eq. 18b}
\]

\[
k_{OFF} = A_{OFF} \times e^{-\frac{E_{OFF}}{RT}} \quad \text{Eq. 19a}
\]

\[
k_{OFF} = \frac{k_{ref}^{OFF}}{e^{-\frac{E_{OFF}}{RT_{ref}}}} \times e^{-\frac{E_{OFF}}{RT}} \quad \text{Eq. 19b}
\]

\[
\ln(k_{ON}) = \text{const} - \frac{E_{ON}}{R} \times \frac{1}{T} \quad \text{Eq. 20a}
\]

\[
\ln(k_{OFF}) = \text{const} - \frac{E_{OFF}}{R} \times \frac{1}{T} \quad \text{Eq. 20b}
\]

From the plot of $\ln(k_{ON})$ vs $1/T$ and $\ln(k_{OFF})$ vs $1/T$ (with $T$ expressed in Kelvin and $R = 8.31 \text{ J mol}^{-1} \text{ K}^{-1}$), the corresponding activation energies ($E_{ON}$ and $E_{OFF}$) can be calculated from the slopes of the linear fits around a restricted interval of temperature around the $T_m$. This
temperature value can be found by the point of intersection of the two linear fits and corresponds to the theoretical melting temperature that would result from cooling or heating the solution mixture at a rate that is infinitely slow. The Arrhenius factors \( A_{\text{ON}} \) and \( A_{\text{OFF}} \) are important to keep the units of measurement equal on both sides of the equation. These values can be obtained from Eq. 18b and 19b at a reference temperature \( T_{\text{ref}} \), once the activation energies are extracted from the slopes of the linear fits.

Once the values of activation energies and Arrhenius factors have been found from the linear plots, the expressions for the rate coefficients can be determined (Eq. 18 and 19) and substituted in Eq. 13, leading to the following system of two ODEs:

\[
\begin{align*}
\frac{d\theta_c}{dt} &= \left( \frac{dT}{dt} \right)^{-1} \left[ A_{\text{ON}} \times e^{-E_{\text{ON}}/RT} \times (1 - \theta_c) - A_{\text{OFF}} \times e^{-E_{\text{OFF}}/RT} \times \theta_c \right] \\
\frac{d\theta_h}{dT} &= \left( \frac{dT}{dt} \right)^{-1} \left[ A_{\text{ON}} \times e^{-E_{\text{ON}}/RT} \times (1 - \theta_h) - A_{\text{OFF}} \times e^{-E_{\text{OFF}}/RT} \times \theta_h \right]
\end{align*}
\]

Eq. 21

Each of the two equations has been solved numerically using a MATLAB code that implements the 4th order Runge-Kutta method with the following initial conditions and parameters:

- **Initial conditions for the cooling process:** \( \theta_c = 0; \ T = 340 \) K, scan rate \( \frac{dT}{dt} = -0.1^\circ\text{C/min} \)
- **Initial conditions for the heating process:** \( \theta_h = 1; \ T = 320 \) K, scan rate \( \frac{dT}{dt} = 0.1^\circ\text{C/min} \)

Finally, we simulated the theoretical equilibrium curve, applying Eq. 24, derived as follows:

\[
K_D = \frac{k_{\text{ON}}}{k_{\text{OFF}}} = e^{-(E_{\text{ON}}-E_{\text{OFF}})/RT} = e^{-\Delta E_{\text{melt}}/RT}
\]

Eq. 22

\[
K_D = \frac{e}{\theta} = \frac{\theta}{1-\theta}
\]

Eq. 23

\[
\theta = \frac{K_D}{1+K_D}
\]

Eq. 24

As expected, whereas the simulation of the heating profile worked quite well, the simulation for the cooling process was not successful. This points out that the unimolecular approximation applied to the folding process of the microdomain nearby the FRET pair(s) is limited. Nevertheless, application of this model enabled to reconstruct a meaningful equilibrium thermal curve (black line in Figure 2c and d of the main manuscript), which was used to estimate the relative thermal stabilities of the two dimers in theoretical equilibrium conditions.
3. On the relative populations of stacking interactions

The experimental data obtained by single-molecule TEM imaging of randomly stacked monomers (Figure S21) indicate the following trend of monomer association: AB = 49%, AA = 28% and BB = 23%. Assuming that the relative populations of stacked interfaces are dictated exclusively by the energy of stacking interactions between the terminal nucleobases of facing monomers ($E_i$), one may apply the Boltzmann distribution to calculate the probability of each mode of interaction to occur ($P_i$). Accordingly:

$$P_i = \frac{e^{-E_i/k_BT}}{Z} = \frac{e^{-E_i/k_BT}}{\sum_i e^{-E_i/k_BT}}$$

Eq. 25

where $Z$ is the sum of all probabilities. Thus, the energies corresponding to the experimental probabilities found by TEM can be obtained by solving Eq. 25 for the three values of $P_i$, as described in the following system of equations:

$$\begin{cases} 
P_{AB} = \frac{e^{-E_{AB}/k_BT}}{Z} \\
P_{AA} = \frac{e^{-E_{AA}/k_BT}}{Z} \\
P_{BB} = \frac{e^{-E_{BB}/k_BT}}{Z}
\end{cases}$$

Eq. 26

with $Z = 1$, $P_{AB} = 0.49$, $P_{AA} = 0.28$ and $P_{BB} = 0.23$. This leads to the following solutions:

$$E_{AB} = -k_BT \ln(P_{AB}) = 0.71k_BT$$
$$E_{AA} = -k_BT \ln(P_{AA}) = 1.27k_BT$$
$$E_{BB} = -k_BT \ln(P_{BB}) = 1.47k_BT$$

with $E_{AB}$, $E_{AA}$ and $E_{BB}$ being, respectively, the stacking energies for formation of the AB, AA and BB interfaces at temperature T. Interestingly, these values of energies are lower than those calculated as the sum of the stacking energies at each interface ($E_{AB(sum)} = -82 k_BT$, $E_{AA(sum)} = -43 k_BT$ and $E_{BB(sum)} = -26 k_BT$). This suggests that, although the trend in energy remains unchanged ($E_{AB} < E_{AA} < E_{BB}$), the simultaneous occurrence of several base stacking interactions in near proximity and/or possible interactions between blunt-end helices that face each other but do not properly touch (i.e. they are separated by 1bp gap) may affect the energy cost needed to establish the dimeric unit.
DNA Sequences

Monomer core:

GCGTTTAATTAAACAGTTCAGAAAGATAGCGCAACACTTTAG
GCTAAAAAGAGTCTTTTACCTGCAGAAGTTCAAAAATAGGAA
AAAGACCACTAAACACCACGTAGGCCAACAAATCGAACCAGAAAT
ATTACCTGAGGTTTGCCTTTAGCCGGCTAGATGCCACTGAG
ATTAAGACGGGTCATTACCATCACCAGGGAGATTTCGGGACG
TAAGCTCTTTTGAATAAGAGCCCGTAGCAACATCGCCTCAT
CTGTTAAATTCATAGGTAGAAAGGTGCTATTAAACAGCAAGA
ACCACTTAAACCGATTGAGAAATAAAGAGAAATTTTCTAGTT
CCCAATAAAAGGCGAAAATGGAATATAAAGCAACGACGCA
CATGAATTATCATCTATATTATACTTTGACTATTTTCTAGTG
CGTAAACCAACCGTCTGGCTAGCCCCGACGAAACAGCGACA
GGGAGGCTATGGGATTAACACCAGACGATTTGCTAACACGAG
GCTTAAAGGCTAGGTTTACCGAGGTAGATTTTACATTGAGAG
AAATCACCTTTTTTGAGAATCTGGAAGTACTATACATAGAG
ACAAATCGCAGAAATCGGATATCCGCTTACATTACTAATG
ATACGTTGCTGAGTTTATGAGATAGTTTAGTTAT
CAATGCACTTTTTTTCGAGAATCTGGAAGTACTATACATAG
ATGAGATTTATGAGATAGTTTAGTTAT
GGCAATTTAAGGCTAGGTTTACCGAGGTAGATTTTACATT
CTGTCAGTTACACGATTAGGCAGGAGGAGGTATACGTGGTTGCT
AACGTGAGCTGAGTTTATGAGATAGTTTAGTTAT
CGGTAGCACTTTTTTGAGAATCTGGAAGTACTATACATAG
AGAATTACCTTTTTCGAGAATCTGGAAGTACTATACATAG
GATTCGTCGTCAGGAAGTTACGCTGAGTTTATGAGATAG
CTTGGAGGTTTATGAGATAGTTTAGTTAT
TTTAGACAGCAAGCGAGCAATAAGCTAAGTAAAACAGG
TTTGGTAGCAAGAAACTAATAAAGAATTAGGAAATAAAGGAG
ATATTCTTCTTTTGAAGAACCTCAATCAATAGGTAAGAGGAC
AGAGGAGGACCTTGATTTTTAAATATGTCGCAAACAGTAAATG
ATTTTACGAGCAATCAATAGAAGAGCGCAGTTGAGACG
TTTGGAGGTTTATGAGATAGTTTAGTTAT
ATTGAAACAAATAGCTATCTTACACGCTAGGTAAGAGGAT
GATTGGGGGCTTTTATGAGATAGTTTAGTTAT
CTTATTCTCCCAGCGTGTAAAGGTCGACGAACTGACAGA
TTTGGGTACGCTGCAAGGCGATTAAATCATGCTTACGCTTTG
TTGCCAGTTTGGGCCTCTCTCTAGATACACATACCGCAAACCTC
AATACCTGAGCAAAAGATTACAAAAAAAAGCAATAAGGAAATTCGGGCGCATGAACGAGCAGCAGGCGAAAATATTGC
CCGATC
CCGAGAACCGTGCTCATAAAATCCCTTATAACAGGGTGCTGT
TTCAGAAGGAATATTCAATCCGGGAAAGAAGAGAAATGTTTTTTA
TTAAGTTTGAGATTAGAGTACCATCGCTAAGGACAAATAAT
CTGGCTCAACCTTTTTTGGCAGTGTGACTCTTGAGGATGCG
GCCCTTTGTGCCGCAAAAGACAAAAACCCAGTAGTTTCAGTGCT
ATCCAACTAAAGGTAAATATTGGGCACCCCGGAAATAAG
CATAATAGCATGAAACACCAGGAGGGTTAGAATGGTTTATACT
TACAACAGGGAAGAAGACTGACCATAAATCAAAATAGTATTAC
ATGCTTTTATCCAGCAGTCGGGAACGTCCACCACCCCT
TCGAGAATAGGAGGCGATTAATCGTAGGCGAGGTTGCACAC
TCTTCAGGCCACCTGTAACATAAAAAACAGATAATTTTGG
TTCCGACCCGTCGTAATATTTTTGACAAACGTAACTACC
CCCTCCCAAGTTGGAACAAACCCGAAATAATCGAACACCCAC
TTGGGTGTTGTGAGATGGGTTAATTTGAGTCCGTC
TACTCGTGCGAGGGAACAGCACATTTGTAAGGAAACCCTTTG
TTACAATATTTTGGAAATACCTACAGCTTTTTAATAGATATAAT
TAATAAGAGATATTTCACTTGGCAGCAGATATATATATATAT
CTCCTTTGACCAAGCTTTAACATGACTTAAAGAAGTAGA
CGAACCCAGAAAGCACGTGTAATGCAGGAGAGATGAGAA
CAGTCTATTTGCGATTTCCATGCTAGTTGGGAGAAAGGT
TAGTAAACCAAGGCAAACGATGAACCGTCGTTATAATCAGCAATA
TATAGGAACTTGTGAATGATAAGAAAAAACAGATCTCTGTC
GTCTAAATGCTCTGACGCAACAGTGGCACGATCACCTCGA
CAGGAAAGATAGAAGGTCAGGTGCTATTAAAGGGATAGGAA
CATGTTTAAATCTACGAAAGCGCATGAAGTGGAGGACTCCCT
CCTCCTTGATTTCAATACATGCTGTTTATAATGCTCAGTACTTAG
CCATAAAATTGCGTTTTTAAGCAGATTTTCTATATCGAGATAAT
AAGAGATGATTTTTTAAATTTTTCAATAACCACATAGCATAG
GATAAAGTTTATCTTTTCAAGTGAAGAT
CGCCATATTTTAAGAAATCTCCAAAAAATAA
ACCTAATTAAAGGAGGCGATTTAAAGGGAGCCGG
AGCATAACACGCTTGGCTCGCTCAGTCCTGCCCCAAAGGG
CAATTTTATCTAGCTTGGAGGTCAACGAGCAGA
GCCCGCCGCGAATCTTTACACGCTAACAGCATTT
CAAACTAAACCGCTAGCTAATGCAAGAAGCAATAAAC
TCGCGTGCTCCTCTTCTATAGCCCGGAATAGGTGAGGGTT
GTAATTGAAGCAGCTTTTAAATAAAGAGAGCGCTTCTTG
TGAGAAGCAGAAAATGAAAAAGGCTTAATACAGGAAGTTGAAT
AGCCTCAGAAGATTGAAAAATTTTTATCTTTAGGAGAACAGA
CGAAAAACCGTGCCCGGATTATTAAGCAATACGCGAAATAA
CGTCGTTTAAAGCTGGGAAAGACGCTGTCGCTG
CTTTGAGTAAAGAAAAACGTAATAGAGGTCCTGAGCTA
TGATAAGCTTTAGTGTAATCAGCGCCGCTTCTGGACAGGAAA
GAGCGCCATTAGCAGCTTCCCCGAAAGCCCAAAAAACATGCA
TTTGTGAACGTGCTTTTCATCGGCATTTTGTCAAGCTATCATACCCGGA
TAGCCGAACCAAAAGAAATACTATTTCAATTAGAGAGATAACCCACAGA
GTCAATCATATGTTAACGGCAGCATGCCGGTAATCTAAAAAGCAATAA
ACGTCGCAAACCGGCAACATTGATCAGGGGAAACGAGCTAGGAAG
AACATGGTTCGTAGCATTCCACTTTTACGTTCAACGCAACATGTAATTT

Hybridization staples:

TTAACAGCTTGATGCCCTGCAACGA
CATTATCTGGCAACAGAGATGAATTA
ATACATAACTCTATTCAATTTACCTTT
AGTGGCACTAACCACACACACACGCAA
CCCTCAAATGCTCAATAAATATTTCCTCAGG
ACTTCTTTGATAGGAAGTCTCAAAATCGGATA
AAAAGAATACACAAAAGACCGGAGAAAAATACCAA
GGTTATATCGTAGTTAATAATCTTTCACATTTCTGC
CTTAAAGTCATGTTCAAAACAGGTAGTTGCTCGGAAC
AAACCTGATTAAAGAGATGAGGTTGCTGAATTACGAA
GGCGAAGTCAAAAGGGTGAGGAAAGCAATGAAAA
TTTAACAGAGGGGAGTTACGGAAATACGAATGCAG
GAAATAACGAGTTAATGAGATTCTAGACATAT
TCGCAAGACTAAACACTCATGGAAGGGAATGCTATAGTG
AAAAAGCATAATGAGGCGAGCCAGTGCAGCTTATGAAAT
TCTTATGGAAGGATGGATGTGTAATGTCCTTATC
GAGGCAATCTCAGTGCAGTACATTGAAAGAGGAGTGCTAC
CCCTCAAATCGTAATAATATTAGGAGGTTAAG
GATCGTCTAGGGAGTTAGGCCCAGAAATCATAATAGATTAAGACGCT

A stacking staples:

GCTTTTGCGAACCAGACC
AGCCTGCCATAGGTGCAC
ACAGATGAAATGAGATGT
GCCAAAAGGTAATATTC
ACGAATGACATGATAAAC
TAAAACTGATTGAAAGG
AAATGAGGGGAGCCAGTGC
TCTTATGGAAGGATGGATGTGTAATGTCCTTATC
GAGGCAATCTCAGTGCAGTACATTGAAAGAGGAGTGCTAC
CCCTCAAATCGTAATAATATTAGGAGGTTAAG
GATCGTCTAGGGAGTTAGGCCCAGAAATCATAATAGATTAAGACGCT
B stacking staples:

TCTGGCCAACCAGCAGCA
AAAGAACGCTAAATCCTT
GAGTCAATAGTGTCGCAAGAC
CGTTGTAGCAATAAGGGACAT
TAACCACACACACGCAAATTAAC
TTCAGGTTTAACAGAGGCGAATTA
AGTACATAAATCAGACCTGAGAATTAAC
TTATATATATTAAGAATAAACA
AATGAAAAACTCTAAGCTGAGACGA
AAACCTGATTTAAGAAATTGCGTAGATT
CAAAATTTATATCATAGATGACGAAT
TTCATTTCAATTACACCAATTTAATGGAAAC
TGCCCGAACGTTATTACTCGTGATGAGAAAAATCCAAAATT
GATGTAAATAACTTCTTTGATTAGAGTCTCAAATCGATA
TATGTCATCCGGCGCGTTAATGCGAATGTTTTTGA
CCGGAATCATATAATGATTTATATATATGTCGCGTACGCGG

Protruding arms for AuNP hybridization:

CAATTGCCCTGATGGTGGTTCCGACAGTGAGAATTCGTAGTTGGGACCGTAAATTATTATTATTATTA
GAATGAGATGGCGGTCCACGCTGGCTGAGAGGCCTGCAACGACGGTAACAACATTATTATTATTATTA
CCGTCCTCATAAAATCCCTTTATAACAGGGTCTGCTGTTTCCGATGTGGTGTATTATTATTATTATTA
GGAATTATTCAGTGTTTGGTTCCAGTATCAGCAGAACATACTCGTGCGAGGGGAATTATTATTATTATTA
GTGGCCCGTAGTGGAATCTCAGGCTGCTGAGGCTGCAACGACGGTAACAACATTATTATTATTATTA
TGCCCCCTGCAAGAGTCCACTATTTCGTGCCAAGTGTACGCAACTGCCTCAGATTATTATTATTATTA
CATGAACGAGCAGCCAGAAATATTGCCCGATCCCCTCCCAGTGAGGGGAAATTATTATTATTATTA
GGCATTAAATGAATAGCCCAGATGGCGGTATTATCCCGCTTACGCCTAAACCCTATTATTATTATTA

Fluorescently labelled staples:

[6FAM]AATGTGAGCGAGCCAGTGCCA
[TAMRA]CGTTGTAGCAATAAGGGACAT
[6FAM]TGCCCGAACGTTATTACTCGTGATGAGAAAAATCCAAAATT
CGTTGTAGCAATAAGGGACAT[TAMRA]
[TAMRA]GGAAGCAAAACTCAGACACCAAGGGGATCGTCAGGAGTTAAAGGCC
AATGTGAGCGAGCCAGTGGCA[6FAM]
References

(1) Castro, C. E.; Kilchherr, F.; Kim, D. N.; Shiao, E. L.; Wauer, T.; Wortmann, P.; Bathe, M.; Dietz, H. A Primer to Scaffolded DNA Origami. *Nat Methods* **2011**, *8*, 221-229.

(2) Pfeifer, W.; Lill, P.; Gatsogiannis, C.; Saccà, B. Hierarchical Assembly of DNA Filaments with Designer Elastic Properties. *ACS Nano* **2018**, *12*, 44-55.

(3) Kaminska, I.; Bohlen, J.; Mackowski, S.; Tinnefeld, P.; Acuna, G. P. Strong Plasmonic Enhancement of a Single Peridinin–Chlorophyll a–Protein Complex on DNA Origami-Based Optical Antennas. *ACS Nano* **2018**, *12*, 1650-1655.

(4) Xia, H.; Bai, S.; Hartmann, J.; Wang, D. Synthesis of Monodisperse Quasi-Spherical Gold Nanoparticles in Water Via Silver(I)-Assisted Citrate Reduction. *Langmuir* **2010**, *26*, 3585-3589.

(5) Sprengel, A.; Lill, P.; Stegemann, P.; Bravo-Rodriguez, K.; Schoneweiss, E. C.; Merdanovic, M.; Gudnason, D.; Aznauryan, M.; Gamrad, L.; Barcikowski, S. *et al.* Tailored Protein Encapsulation into a DNA Host Using Geometrically Organized Supramolecular Interactions. *Nat Commun* **2017**, *8*, 14472.

(6) Mergny, J. L.; Lacroix, L. Analysis of Thermal Melting Curves. *Oligonucleotides* **2003**, *13*, 515-537.

(7) Protozanova, E.; Yakovchuk, P.; Frank-Kamenetskii, M. Stacked–Unstacked Equilibrium at the Nick Site of DNA. *J Mol Biol* **2004**, *342*, 775-785.