

Supplementary Materials for

**Algorithmic design of DNA nanostructures with non-planar curved helices**

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# Supplementary Information

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# S1 ENSnano geometric model to design curved DNA helix bundles, Table ST1, algorithm SA1, and figs. S1 to S13

## S1.1 Default DNA parameters used in ENSnano

The 3D positions of Nucleotides are computed in ENSnano using six parameters as shown in fig. S1: the helix radius  $R$ , the rise  $\Delta$ , the inclination  $I$ , the helicity  $\chi$ , the minor groove angle  $\alpha$  and the inter-helices axis distance  $H$ . The default value of these parameters in table ST1 are based on three papers: the crystallographic data from<sup>23</sup> for  $R$ ,  $\Delta$ ,  $I$ , and  $\alpha$ ; data from<sup>22</sup> for  $\alpha$  and  $\chi$ ; and data from<sup>34</sup> for  $H$ . Phosphate positions parameters from<sup>23</sup> are used to compute the nucleotides positions parameters by placing the nucleotides in the middle of two consecutive phosphates: as a consequence, the nucleotide inclination is set to  $I_N = I_P - 2 \times (\Delta/2) = 0.375 - 0.332 = 0.043$  nm and the minor groove angle is set to  $\beta_N = \beta_P - 2 \times (\alpha/2) = 170.4^\circ - 34.48^\circ = 135.92^\circ$ . The inter-helix axis distance is set to  $H = 8\Delta = 2.65$  nm based on the double-layer origami data from<sup>34</sup> as the inter-helix distance from one layer corresponds to the distance spanned by 8 bases pair in the other layer. This value is moreover consistent with the average inter-helix distance reported in<sup>6</sup>.

Note that the most important parameters for DNA origami design are  $\Delta$ ,  $\alpha$ ,  $\chi$  and  $H$  as any error on them will accumulated over the span of the design (as opposed to  $R$ ,  $\beta$ , and  $I$ ). As a fortunate consequence, they are thus the easiest to adjust if needed, as any error on them would be easily detected in experimental data. Finally, recall that it is always possible to impose your own DNA parameters in the ENSnano .ens file, as these may change with your own experimental setting (buffer,...).

|                                            | Phosphate positions <sup>23</sup>                                           | Nucleotide positions   |
|--------------------------------------------|-----------------------------------------------------------------------------|------------------------|
| Radius                                     | $R = 0.93$ nm                                                               |                        |
| Rise                                       | $\Delta = 0.332$ nm                                                         |                        |
| Inclination                                | $I = 0.375$ nm                                                              | $I = 0.043$ nm         |
| Twist ( $\alpha$ ) and Helicity ( $\chi$ ) | $\alpha = \frac{360^\circ}{\chi} = 34.48^\circ$ with $\chi = 10.44$ bp/turn |                        |
| Minor groove angle                         | $\beta = 170.4^\circ$                                                       | $\beta = 135.92^\circ$ |
| Inter-helices axis distance                | $H = 2.65$ nm                                                               |                        |

Table ST1: DNA double-helix parameters used in ENSnano: the nucleotides positions are deduced from the phosphate positions as measured in<sup>23</sup> and the DNA parameters used in<sup>22,34</sup>.

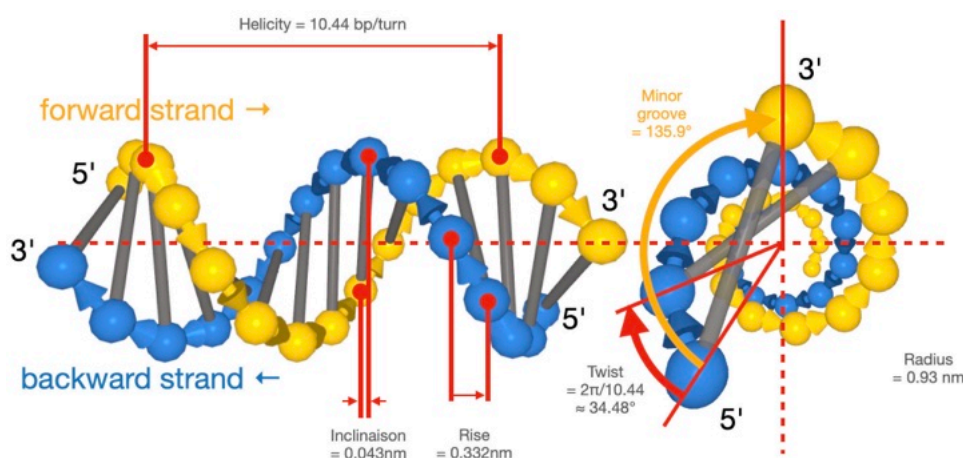


Figure S1: Illustration of DNA double helix parameters.

## S1.2 Curvature and torsion of a 3D curve

Given a 3D curve  $t \mapsto M(t)$ , we denote by  $\dot{M}(t)$ ,  $\ddot{M}(t)$  and  $\dddot{M}(t)$  the 1st, 2nd and 3rd derivatives of  $M(t)$  with respect to  $t$ . The *curvature* at  $M(t)$  is defined as:

$$\gamma(t) = \frac{\|\dot{M}(t) \wedge \ddot{M}(t)\|}{\|\dot{M}(t)\|^3}$$

Its inverse  $R(t) = 1/\gamma(t)$  is the *curvature radius* at  $M(t)$ , that is the radius of its *osculating circle*, which is the circle best approximating the curve at  $M(t)$ . The *torsion*  $\tau(t)$  at  $M(t)$  measures how fast the curve takes off from the plane of its osculating circle. It is given by the formula:

$$\tau(t) = \frac{\det[\dot{M}(t), \ddot{M}(t), \dddot{M}(t)]}{\|\dot{M}(t) \wedge \ddot{M}(t)\|^2}$$

It is measured in  $\text{nm}^{-1}$ .

Curvature and torsion at  $M(t)$  characterizes the *osculating helix*, that is the helix best matching the curve locally at  $M(t)$ . Torsion is thus a natural parameter to measure when dealing with DNA helices. Refer to<sup>25</sup> for full details.

As DNA helices are not continuous mathematical curves but discrete, made of nucleotides organised in helicoidal configuration, we use the following discrete version of the torsion. If  $M(i)$  be the positions of consecutive nucleotides in a DNA strand, we define the 1st, 2nd, and 3rd *discrete derivatives* at  $M(i)$  as:

$$\Delta M(i) = M(i+1) - M(i),$$

$$\Delta^2 M(i) = \Delta M(i+1) - \Delta M(i) = M(i+2) - 2M(i+1) + M(i),$$

$$\text{and } \Delta^3 M(i) = \Delta^2 M(i+1) - \Delta^2 M(i) = M(i+3) - 3M(i+2) + 3M(i+1) - M(i).$$

The *discrete torsion* is then defined as:

$$\frac{\Delta}{\tau}(i) = \frac{\det[\Delta M(i), \Delta^2 M(i), \Delta^3 M(i)]}{\|\Delta M(i) \wedge \Delta^2 M(i)\|^2}$$

The discrete torsion of a straight DNA helix is constant to:

$$\frac{\Delta}{\tau}_{\text{straight DNA helix}} \doteq 0.4851 \text{ nm}^{-1}$$

Next, we will use discrete torsion to evaluate the quality of our algorithms mapping DNA onto arbitrary 3D curves.

### S1.3 Curved DNA model in ENSnano

Our geometric curved DNA helix model relies on the definition of a moving frame  $\mathbf{F}(t) = (\mathbf{X}(t), \mathbf{Y}(t), \mathbf{Z}(t))$  that adaptively follows the intended curve  $t \mapsto M(t)$  (Fig. 1A). The  $i$ -th nucleotides from the forward and backward strands are placed around the curve using the frame  $\mathbf{F}(t)$ . Their positions  $N_i^f$  and  $N_i^b$  are computed from the helix axis points  $M(t_i^f)$  and  $M(t_i^b)$  located at curvilinear distances  $i \Delta$  and  $(I + i \Delta)$  from the origin of the curve, where  $\Delta$  and  $I$  are the rise and inclination of the DNA B-form helix<sup>23</sup> using the following formula:

$$N_i^f = M(t_i^f) + R \cos(\rho + i \alpha) \mathbf{X}(t_i^f) + R \sin(\rho + i \alpha) \mathbf{Y}(t_i^f)$$

$$N_i^b = M(t_i^b) + R \cos(\rho + i \alpha + \beta) \mathbf{X}(t_i^b) + R \sin(\rho + i \alpha + \beta) \mathbf{Y}(t_i^b)$$

where  $s(t) = \int_0^t \|\dot{M}(t)\| dt$  is the curvilinear abscissa (with  $\dot{M}(t) = \frac{dM(t)}{dt}$ ),

and  $t_i^f = s^{-1}(i \Delta)$  and  $t_i^b = s^{-1}(I + i \Delta)$  are the parameters  $t$  for the projections of the  $i$ -th nucleotides (forward and backward) on the curve,

and  $R$ ,  $\alpha$ ,  $\beta$ , and  $\rho$  are respectively the radius, the helical twist, the narrow groove angle, and the roll of the DNA helix (table ST1 and fig. S1).

Several approaches exist for defining a frame  $F(t)$  along a curve. The classically used is the Frenet frame, which captures important geometrical properties of the curve such as the tangent, the normal, the osculating plane, etc. However, the Frenet frame tends to undergo sudden rotations in order to adapt to the osculating plane<sup>25</sup>. Using this frame leads to unrealistically twisted DNA helices, ignoring the prevalence of the base-pair stacking interactions which tends to enforce a constant twist around the DNA helix axis (fig. S3).

We thus introduce a *lazily adaptive frame*  $\mathbf{F}(t) = (\mathbf{X}(t), \mathbf{Y}(t), \mathbf{Z}(t))$  that rotates as little as possible along the curve.  $\mathbf{Z}(t)$  is the normalized tangent to the curve at  $M(t)$  for all  $t$ .  $\mathbf{X}(t)$  and  $\mathbf{Y}(t)$  are built incrementally along the curve at each of the required locations  $t_i^f, t_i^b$  according to algorithm SA1.

### S1.4 Adaptive moving frame algorithm

We denote by  $\text{Normalized}(U) = U/\|U\|$  the normalized vector of a non-zero vector  $U$ . For two non-colinear vectors  $u$  and  $v$ , we denote by  $\text{rot}_{u \rightarrow v}$  the rotation mapping  $u$  to  $v$  around the axis perpendicular to both  $u$  and  $v$  (i.e., around the cross product  $u \wedge v$  of  $u$  and  $v$ ).

Algorithm SA1 constructs the adaptive frames used to embed DNA helices in 3D along a given curve in ENSnano.

---

#### Algorithm SA1: Lazily adaptive frame along a parameterized curve $M(t)$

---

**Input:** A parameterized curve  $t \mapsto M(t)$  and a set  $T = \{t_i^f\} \cup \{t_i^b\}$  of the parameters where we need a frame

**Output:** An adaptive right-handed frame  $\mathbf{F}(t) = (\mathbf{X}(t), \mathbf{Y}(t), \mathbf{Z}(t))$  for  $t \in T$ .

*/\* Initialization \*/*

**sort**  $T = \{t_1 < \dots < t_{2n}\}$

**forall**  $k = 1..2n$  **do**

**set**  $\mathbf{Z}(t_k) := \text{Normalized}(\dot{M}(t_k))$     */\*  $\mathbf{Z}(t_k)$  is the normalized tangent \*/*

$\mathbf{X}(t_1)$  and  $\mathbf{Y}(t_1)$  are set arbitrarily so that  $(\mathbf{X}(t_1), \mathbf{Y}(t_1), \mathbf{Z}(t_1))$  is a right-handed frame, e.g.: **choose**

$\mathbf{U} \in \{(1, 0, 0), (0, 1, 0), (0, 0, 1)\}$  such that  $\mathbf{U}$  is not colinear to  $\mathbf{Z}(t_1)$ , and **set**

$\mathbf{Y}(t_1) := \text{Normalized}(\mathbf{Z}(t_1) \wedge \mathbf{U})$  and  $\mathbf{X}(t_1) := \mathbf{Y}(t_1) \wedge \mathbf{Z}(t_1)$

*/\* Adaptive frame computation \*/*

**for**  $k = 2, 3, \dots, 2n$  **do**

**set**  $\rho_k = \text{rot}_{\mathbf{Z}(t_{k-1}) \rightarrow \mathbf{Z}(t_k)}$     */\* the rotation mapping  $\mathbf{Z}(t_{k-1})$  to  $\mathbf{Z}(t_k)$  \*/*

**set**  $\mathbf{X}(t_k) := \rho_k(\mathbf{X}(t_{k-1}))$  and  $\mathbf{Y}(t_k) := \rho_k(\mathbf{Y}(t_{k-1}))$     */\* rotate the frame according to  $\mathbf{Z}(t_k)$  \*/*

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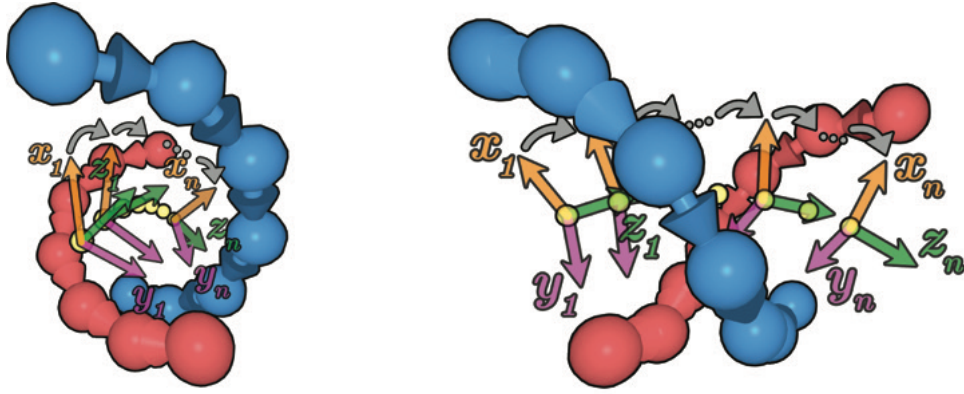


Figure S2: The moving frames  $\mathbf{F}_k = (\mathbf{X}_k, \mathbf{Y}_k, \mathbf{Z}_k)$  is computed iteratively along the curve (yellow dots) such that  $\mathbf{Z}_k$  is tangent to the curve. The nucleotides of the DNA helix following this curve are then positioned according to this moving frames.

## S1.5 Adaptive moving frame along a curve

As shown in fig. S3, our algorithm SA1 produces a smoother embedding of the DNA double helix embedding than the classic Frenet frame. Indeed the Frenet frame is very sensitive to any local change in curvature and tends to rotate erratically as can be observed in fig. S3(left), inducing unwanted torsion in the DNA helix mapping, while our adaptive frame keeps a consistent orientation along the whole curve, minimizing the overall torsion along the DNA strand as can be observed in the lower part of the figure.

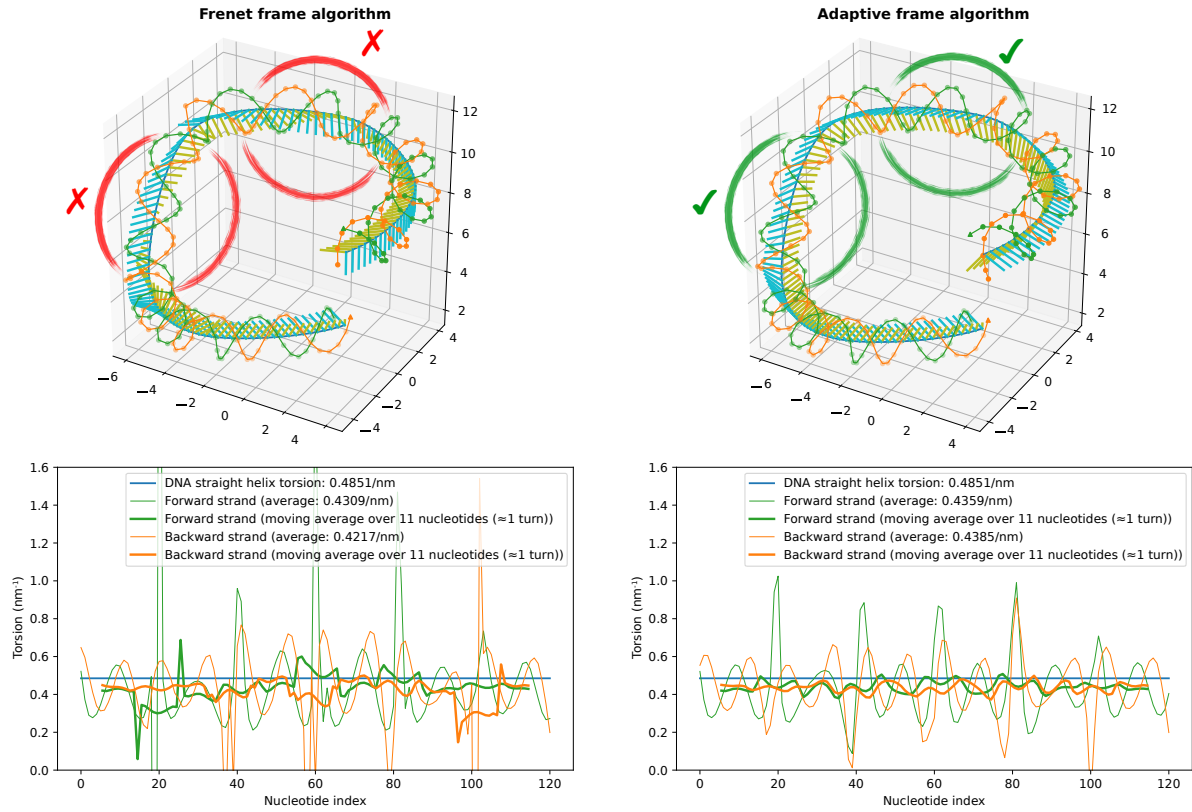


Figure S3: Frenet vs adaptive moving frame along a curve: the curve is shown in dark blue and the computed frames at each nucleotide position are shown in green (X) and cyan (Y) (Z is not shown for clarity as it is always tangent to the curve). At the bottom: the values of the discrete torsion of the two forward and backward DNA strands in both models; in bold, the moving average taken over a full turn of each DNA strand shows that the iterative frame produces a smoother torsion whose value is closer to the torsion of a straight DNA helix.

## S1.6 Comparison of ENSnano curved DNA helix model with the human nucleosome core particle 2CV5 crystallographic data

Figures S5 and S6 evaluate the discrepancy between the DNA strands 1) as placed in space by ENSnano algorithm using the trajectory of the axis of the DNA helix in the human nucleosome core particle crystallographic data PDB-2CV5, and 2) as placed in the PDB file. Figure S6 compares the positions of the phosphates in both ENSnano model and crystallographic data. Figure S6 compares the positions of the nucleotides in both ENSnano model and crystallographic data. We observe a discrepancy of  $0.444 \pm 0.219$  nm for the phosphates and  $0.540 \pm 0.231$  nm for the nucleotides. Note that the DNA helix in the nucleosome core particle is constrained to adopt its shape by its interactions with the histones at its core, interactions which are not taken into account by ENSnano which relies on the sole trajectory of the helix to place the DNA strands.

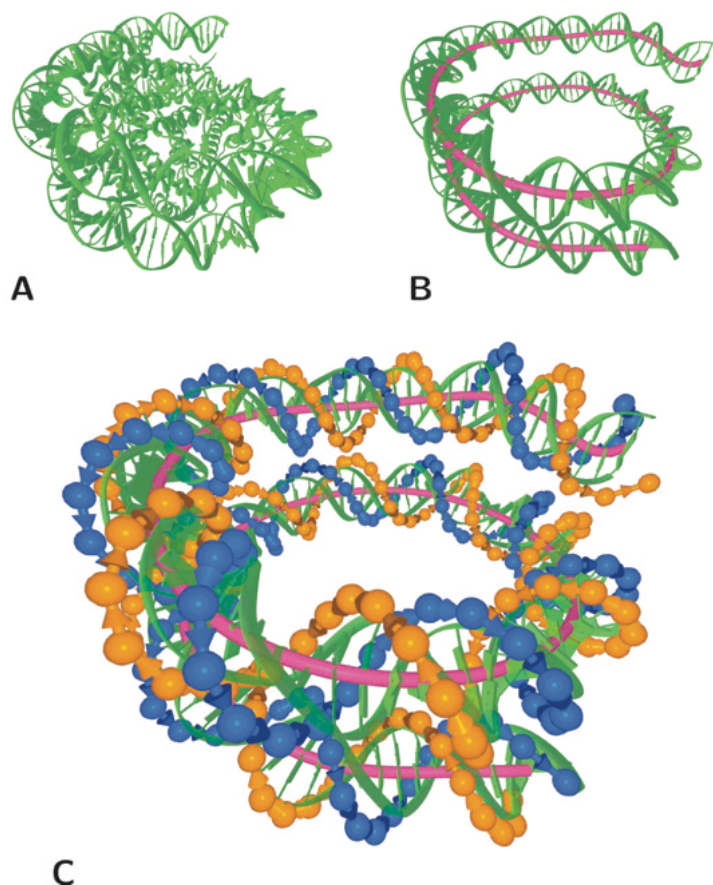
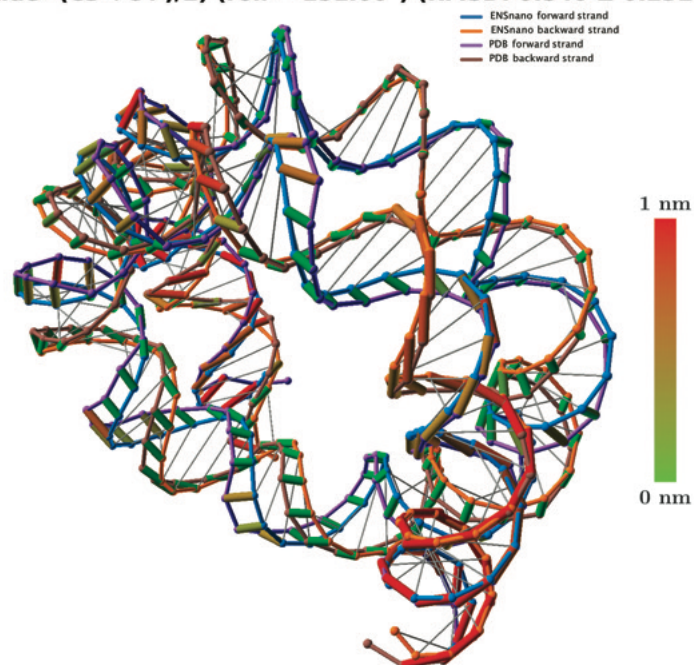


Figure S4: Curved DNA helix model generated by ENSnano from the crystallographic data of nucleosome. A) Crystallographic data PDB-2CV5; B) DNA helix axis trajectory  $T$  (in pink) deduced from the PDB file; C) Curved DNA helix generated by ENSnano from the trajectory  $T$  compared to the crystallographic data PDB-2CV5.

**Nucleotides positions in ENSnano and PDB files**  
**(Nucleotide=(C3'+C4')/2) (roll = 151.00°) (RMSD: 0.540 ± 0.231 nm)**



**Distance in nm between nucleotides in ENSnano and PDB files**  
**(Nucleotide=(C3'+C4')/2) (roll = 151.00°) (RMSD: 0.540 ± 0.231 nm)**

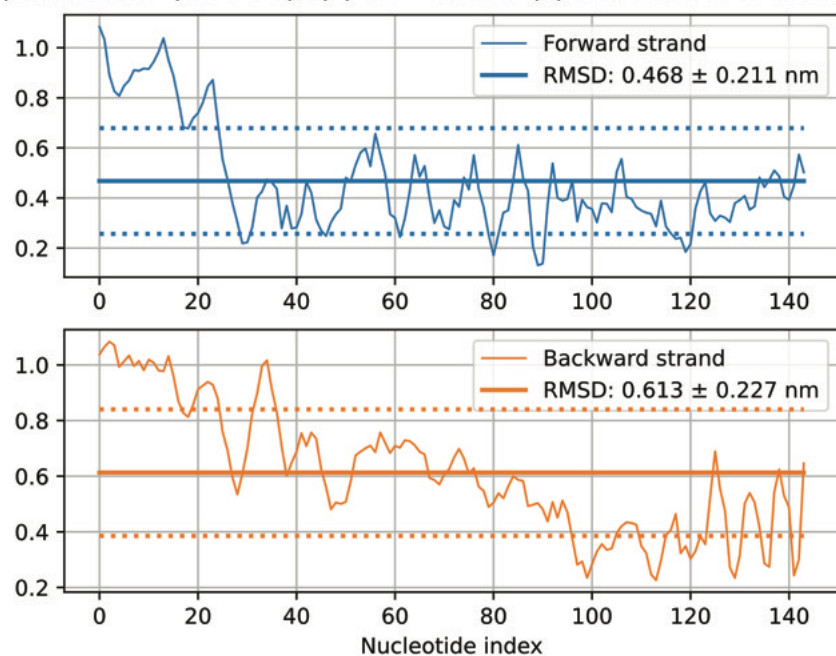
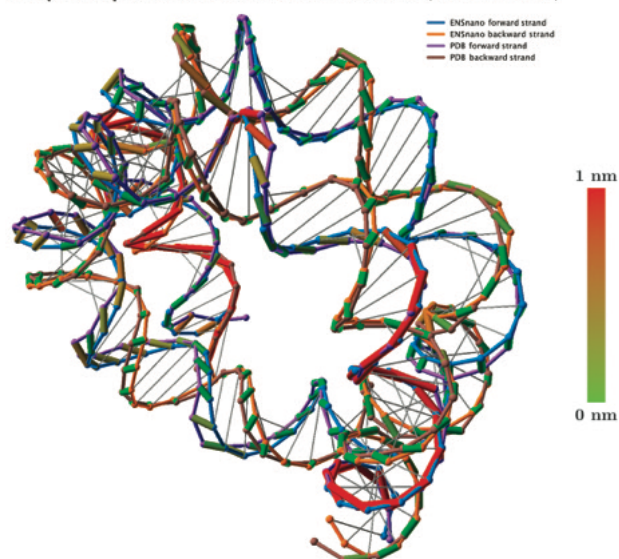


Figure S5: Our curved DNA helix model vs crystallographic data of nucleosome.



Phosphates positions in ENSnano and PDB files (roll = 134.50°)



Distance in nm between phosphates in ENSnano and PDB files (roll = 134.50°) (RMSD: 0.444 ± 0.219 nm)

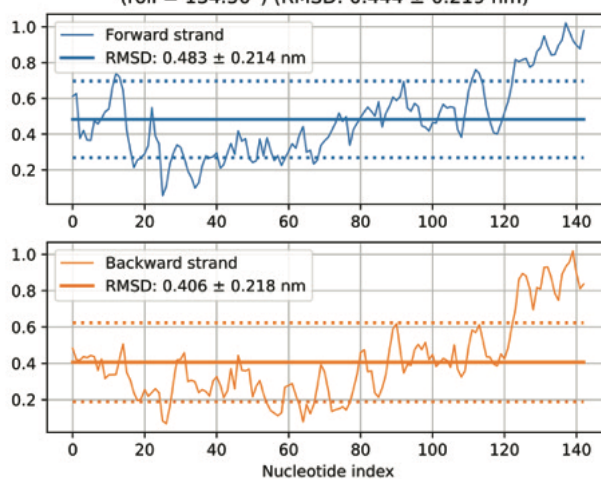


Figure S6: Our curved DNA helix model vs crystallographic data of nucleosome.



## S1.7 Curved DNA bundles

Curved DNA bundles are defined in ENSnano by attaching a grid to a Bézier curve as shown in fig. S7. The frames computed along the curve by algorithm SA1 are used to orient the grid. The grid can be either squared or honeycomb and has edges of length  $H$  corresponding the target inter-helix axis distance (2.65 nm by default). The trajectory of each helix in the bundle simply follows the trajectory of the corresponding point in the grid along the curve (fig. S7B). To avoid self-collision of the origami design, each grid can be moved vertically at the control points of the Bézier curve as we did in our  $\mathbb{X}$ -origami (fig. S7D).

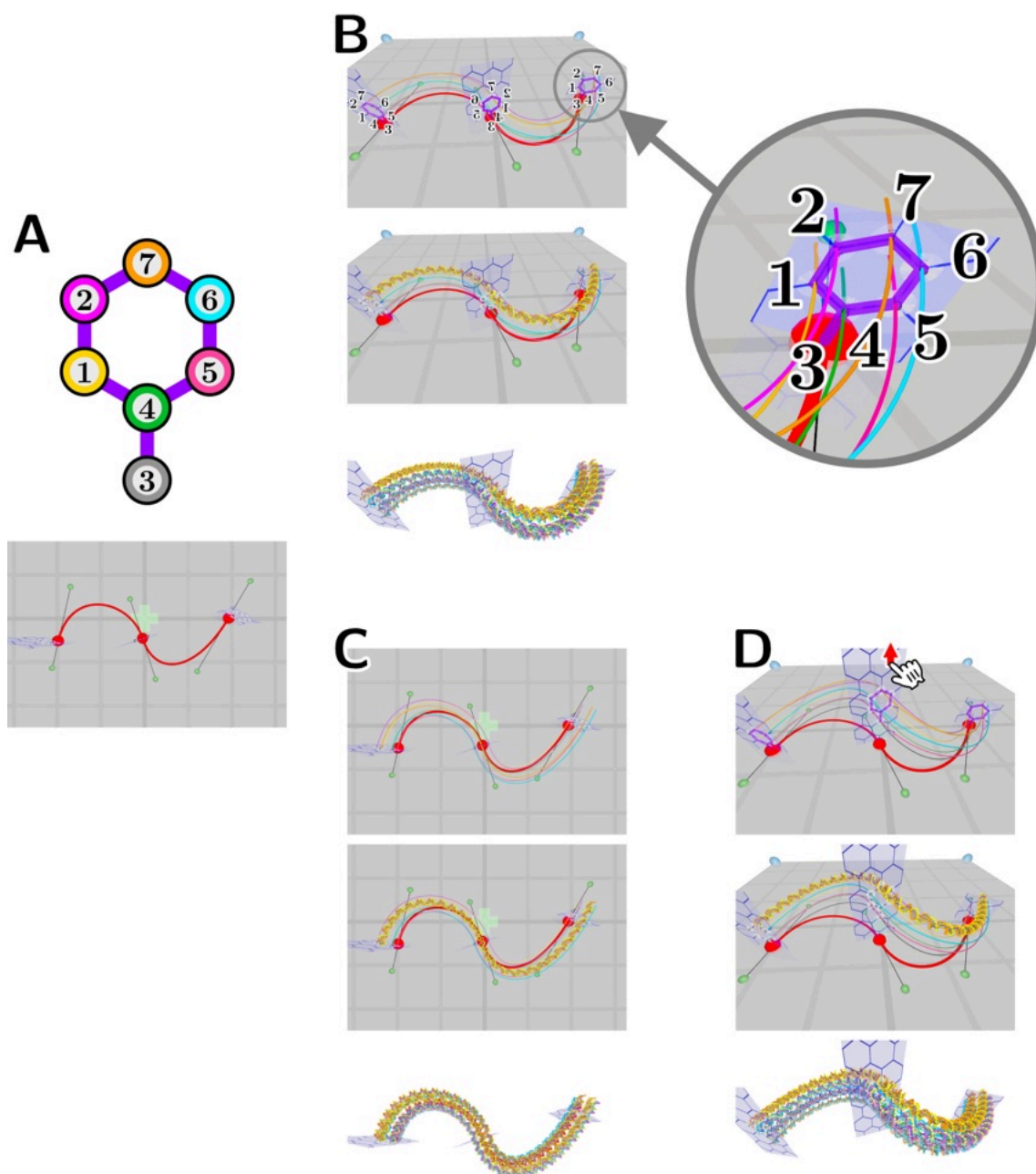


Figure S7: Grids-based routing of helices in curved DNA bundles: A) the helices' positions on the honeycomb grid; B) the trajectories of each helices is deduced by translating every point of the curve according to the grid; C) the resulting DNA bundle; D) grid can be moved vertically at Bézier control points, for instance to avoid self-collision.

## S1.8 2D embedding of curved DNA bundles

In ENSnano, as explained above, the trajectories of DNA-bundle helices are generated by translating the points of a Bézier curve according to an attached grid (fig. S7). The grid defined along the reference Bézier curve is then used as a sectional coordinate system to align, in the 2D view, nucleotides belonging to the same cross-sectional position across the translated helices (fig. S8).

ENSnano consequently provides a 2D representation in which nucleotides from all helices in the bundle are automatically aligned, facilitating both crossover placement and structural inspection (fig. S9). Notably, even when helices within the bundle have substantially different lengths, nucleotide alignment is preserved without requiring the user to introduce insertions or deletions, as is typically necessary in caDNAno.

More specifically, ENSnano achieves this by dynamically adapting the width of each nucleotide cell in the 2D view (fig. S8): cells are automatically enlarged for shorter helices and compressed for longer ones. Thanks to the high performance of ENSnano, this process is instantaneous, and the views update in real time as the Bézier curve is edited.

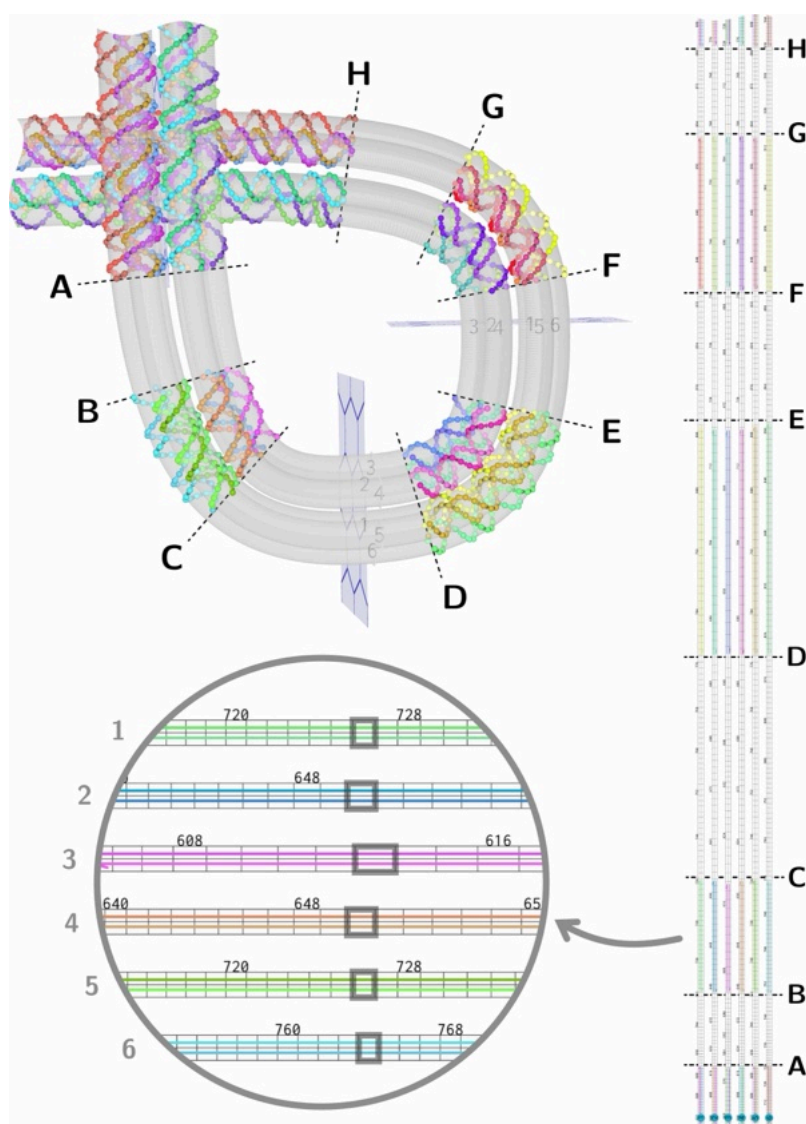


Figure S8: The 2D view automatically adjusts nucleotide cell widths to account for bundle curvature, ensuring that nucleotides occupying the same cross-sectional position remain aligned across helices. Consequently, even helices with substantially different lengths can be represented coherently. For example, nucleotides 613 on helix 3 and 764 on helix 6 correspond to the same bundle section and are therefore displayed at the same position in the 2D view.

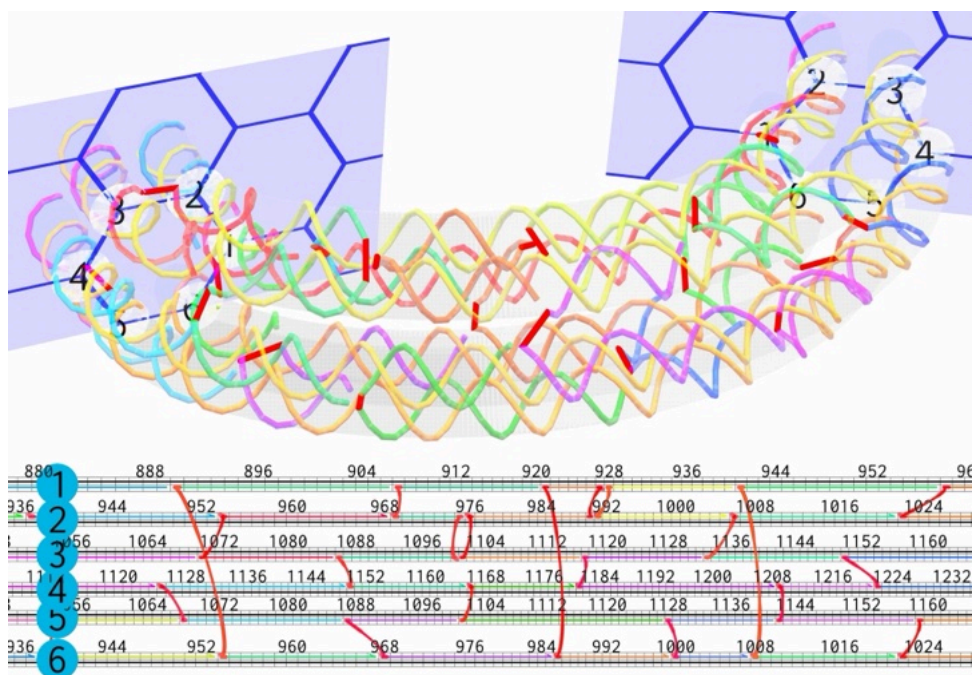


Figure S9: In the figure, all crossovers are highlighted in red in both the 2D and 3D views. By automatically adapting the 2D representation, ENSnano maintains crossover alignment across helices of different lengths, resulting in a precise, compact and readable representation without the need for manual insertions or deletions.

## S1.9 DNA parameters adjustment for closed curves

Closed (curved) DNA helices require special care as their strands need to wrap upon themselves, see fig. S10. DNA bundles require furthermore adjustment and will be handled in the next section. Single closed DNA helices are handled in a three steps process that aims at spreading the constraints evenly over the whole curve:

1. *Tune the rise*: the first step consists in tuning minimally the rise so that the length of curve corresponds to an integral number of base pairs. Let  $L$  be the length of the Bézier curve. Let  $N = \lfloor L/\Delta \rfloor$  be the rounded number of base pairs fitting into the curve. We set  $\tilde{\Delta} = L/N$  be the adjusted rise of DNA for this curve. Note that  $\tilde{\Delta}$  differs from  $\Delta$  by at most  $\Delta/2N$  which is a few ‰ as soon as  $N \gtrsim 200$ .
2. *Compute the frames*: Use algorithm SA1 to compute the frames at every position and map the DNA strands along the curve accordingly.
3. *Tune the helicity*: the last step consists in aligning the angle of both ends of the strands, see fig. S12. Let  $\Theta \in [-\pi, \pi)$  denotes the angle that the virtual  $N + 1$ -th nucleotide makes with the 1st of the strand. We set  $\tilde{\alpha} = \alpha - \Theta/N$  so that the angle difference gets compensated little by little over the whole length of the strand bringing both ends together. Note that, again, the relative difference between  $\tilde{\alpha}$  and  $\alpha$  is less than  $\pi/N$  which accounts for a few ‰ as soon as  $N$  is larger than a few hundred. Equivalently, we set  $\tilde{\chi} = 1/(1/\chi - \Theta/N)$  so that  $2\pi N/\tilde{\chi} = 2\pi N/\chi - \Theta$ .

This process is motivated by the fact that we believe that the constraints on the rise (optimized first) are tighter than the constraints on the twist (optimized second) which can be absorbed by the DNA flexibility as was demonstrated by the variety of origamis which were designed and assembled successfully with helicity ranging from 10.2 to 10.8 base pairs per turn. Note that these adjustments are below the currently known precision on these parameters as soon as  $N \gtrsim 500$ , that is for strands of  $\gtrsim 150$  nucleotides-long.

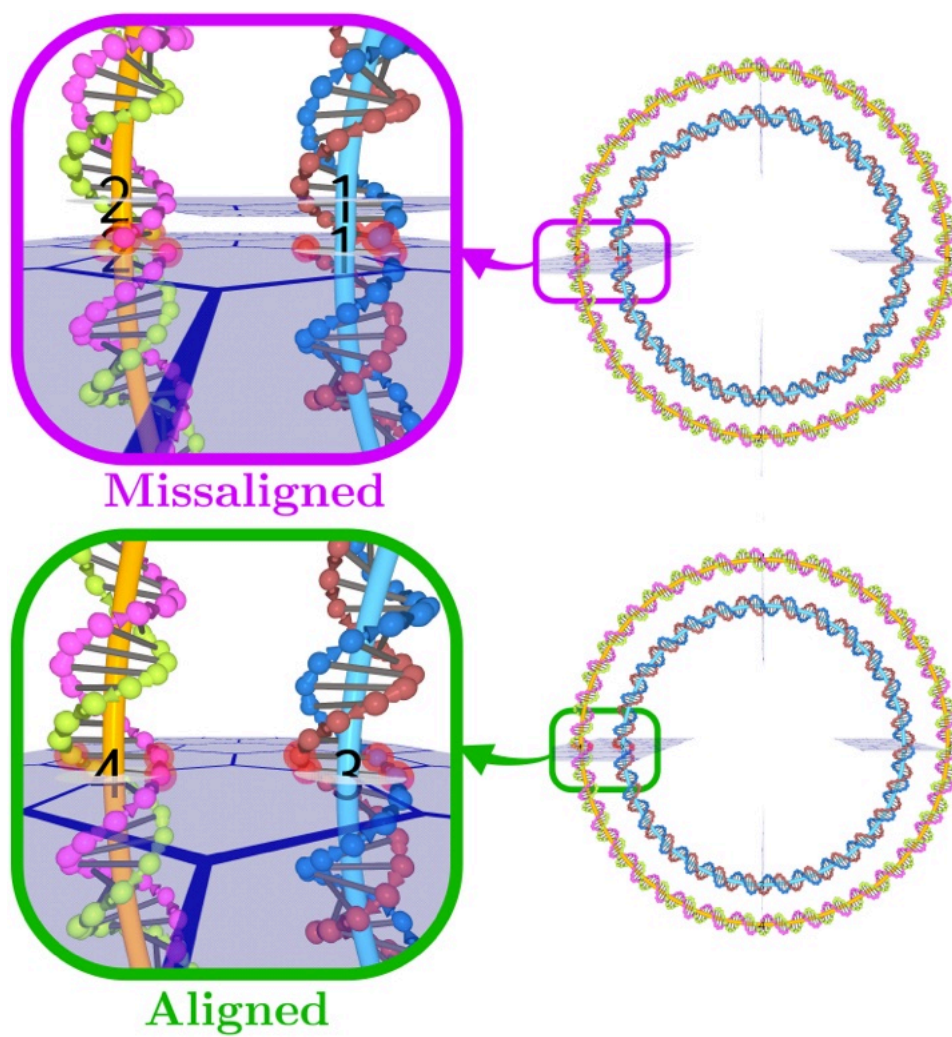


Figure S10: Rounding closed curve's length, twist so that both of its ends match



DNA helix bundles require furthermore adjustments of the parameters because in order for both ends of shifted curves to match, we need to align also the initial and final frames as shown in fig. S11. We proceed by modify Step 2 as follows:

2'. *Compute and adjust the frames for the Bézier curve supporting the DNA bundle:* Use algorithm SA1 to compute the frames at every position. Let  $\Theta \in [-\pi, \pi)$  be the angle between the virtual  $N + 1$ -th frame and the 1st. For  $i = 1..N$ , rotate the  $i$ -th frame  $F_i$  by  $-i \times \Theta/N$  around its  $Z$  axis to absorb  $\Theta$  (see fig. S11). Interpolate using piecewise Bézier curves or Chebyshev polynomials the trajectories of the grid-points by which passes the helices of the bundle, as illustrated in fig. S11)(bottom). Finally, run the three steps in the section above for each of these trajectories as shown in fig. S12.

Note that each helix of the closed-curved bundle has its own adjusted rise and twist (or helicity). The resulting helix 3D embedding is shown in fig. S13.

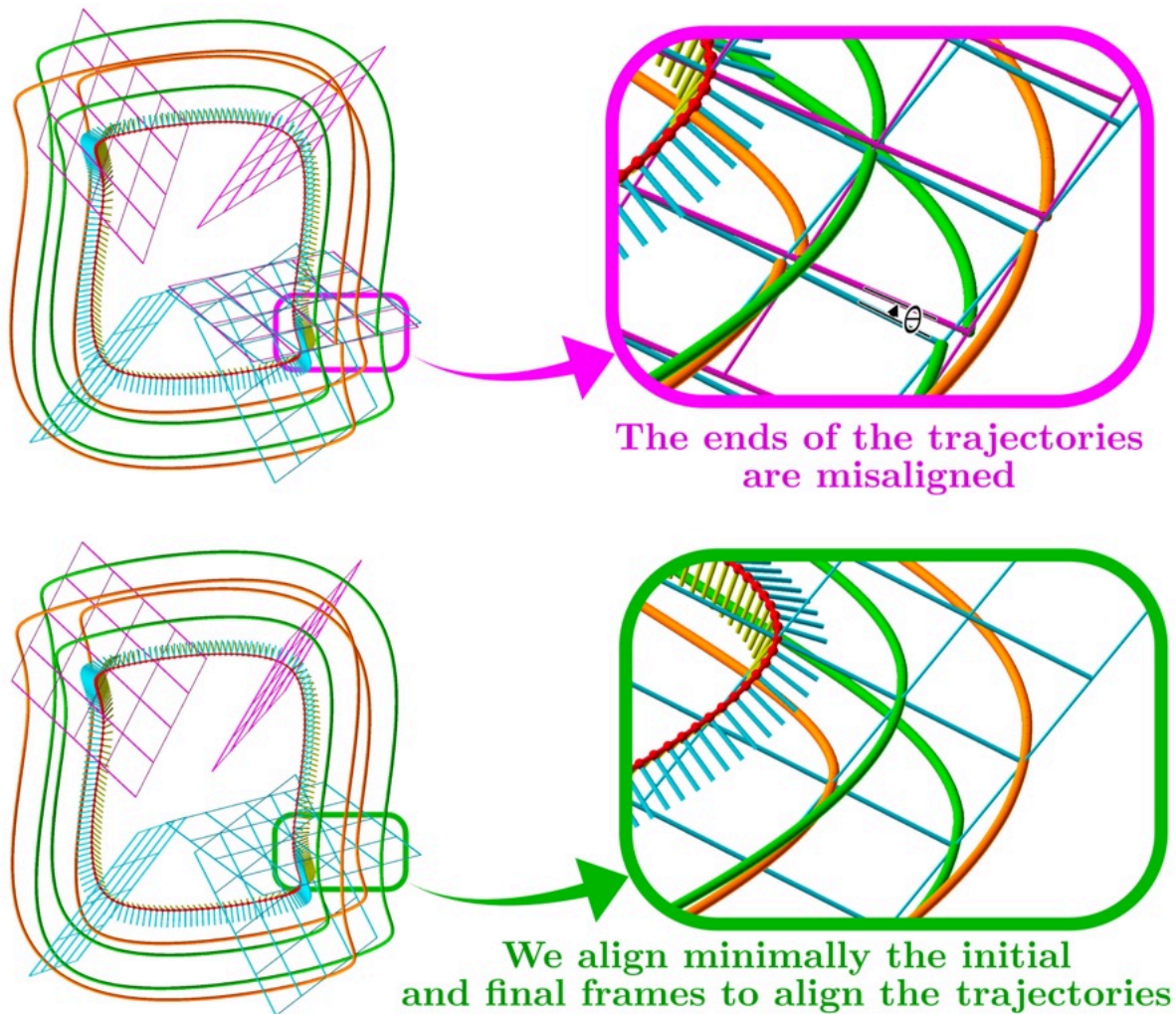


Figure S11: The case of closed curve bundle: 1) Adapt the DNA helix' rise so that the curve's length corresponds to an integer number of base pairs, then compute the iterative frames and rotate them progressively and minimally so that both of initial and final frames match

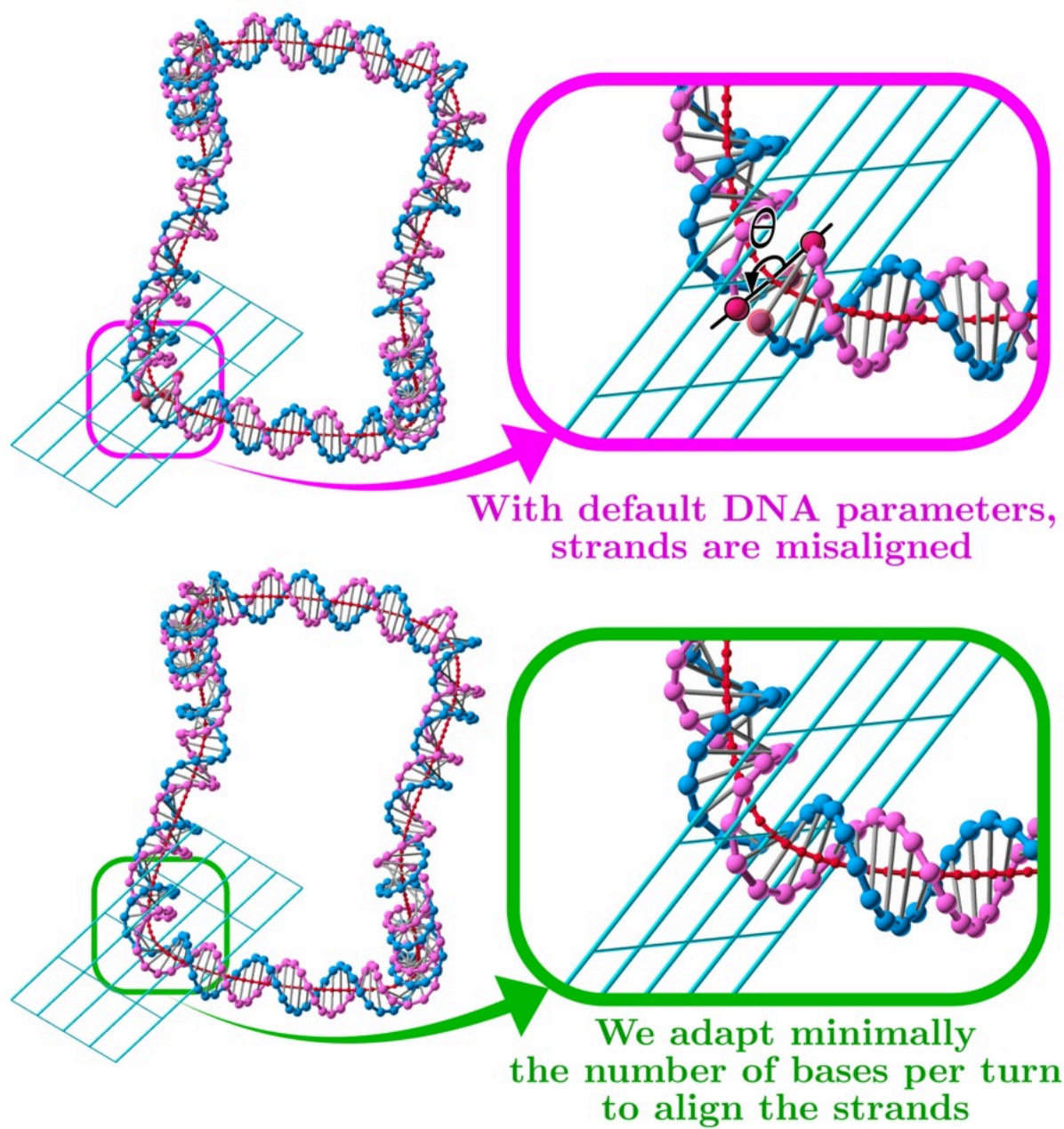


Figure S12: The case of closed curve bundle: 2) Adapt the helicity parameter minimally so that the ends of both strands align.

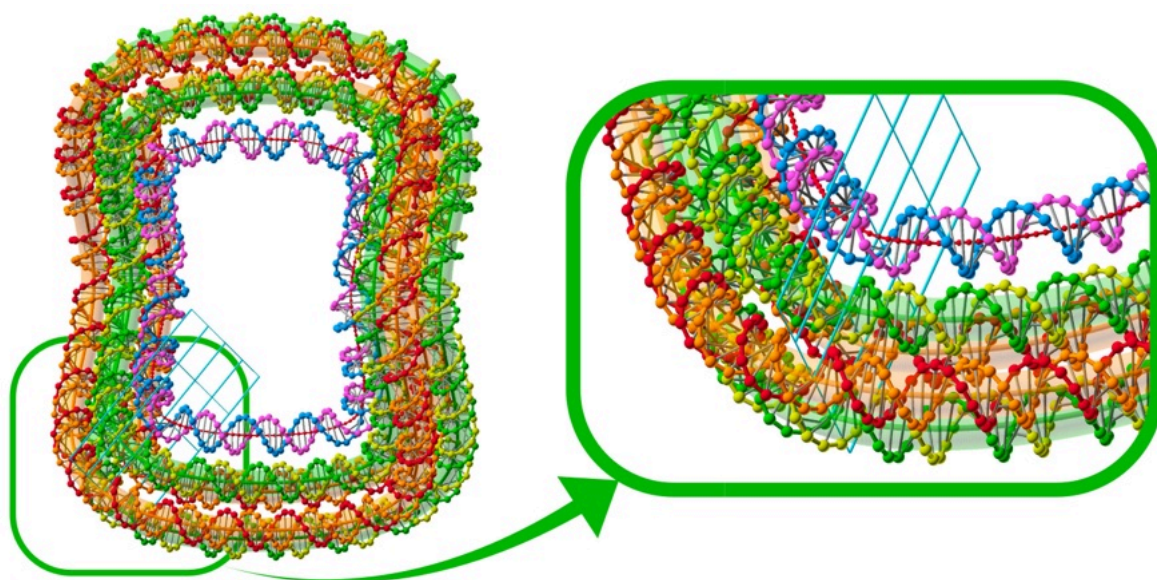


Figure S13: The case of closed curve bundle: 3) Finally, compute the frames and adapt the helix parameters as before for each shifted helix independently, resulting in closed DNA helices respecting the desired inter distance everywhere.



## S2 A four-loop bundle, Figures S14 to S41

### S2.1 Designing the four-loop bundle in ENSnano

Curved DNA bundles can be designed in ENSnano in six steps:

1. Draw a Bézier curve in a dedicated Bézier plane.
2. Attach a square or honeycomb grid to the curve.
3. Add DNA helices to the grid.
4. For non-planar bundles, adjust the position and orientation of the grid in three dimensions.
5. Scale the design to the desired size using the built-in length estimator.
6. Place crossovers interactively in either the 2D or 3D view.

Figure S14 provides a detailed overview of the ENSnano graphical interface for designing curved DNA bundles.

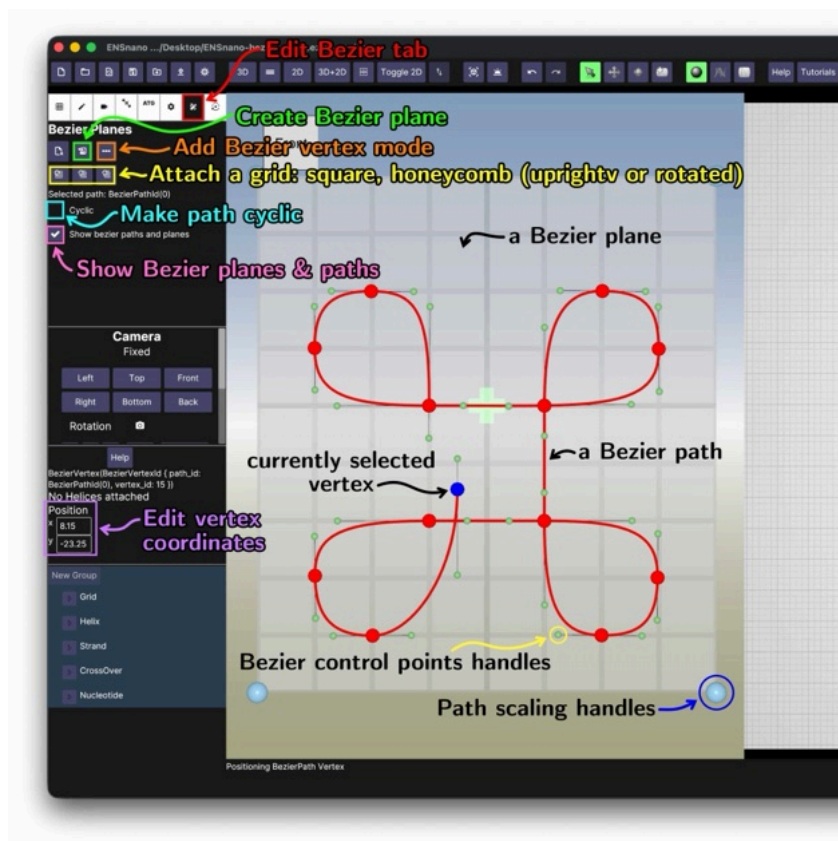


Figure S14: ENSnano Bézier-path design interface. (1) Select the “Edit Bézier” tab. (2) Create a Bézier plane and switch to “Add Bézier Vertex” mode. (3) Place Bézier vertices and adjust their tangent handles to define the path; vertex coordinates can also be specified directly through the context panel. (4) Specify whether the path is open or cyclic. (5) Use the plane handles to rotate and scale the selected path. Bézier paths and planes can be hidden from the 3D view using the corresponding checkbox in the “Edit Bézier” tab.

1139 **Interactive design of curved DNA bundles in ENSnano.** Figures S15 to S17 presents the step-by-step  
 1140 design of the four-loop DNA origami structure in ENSnano.

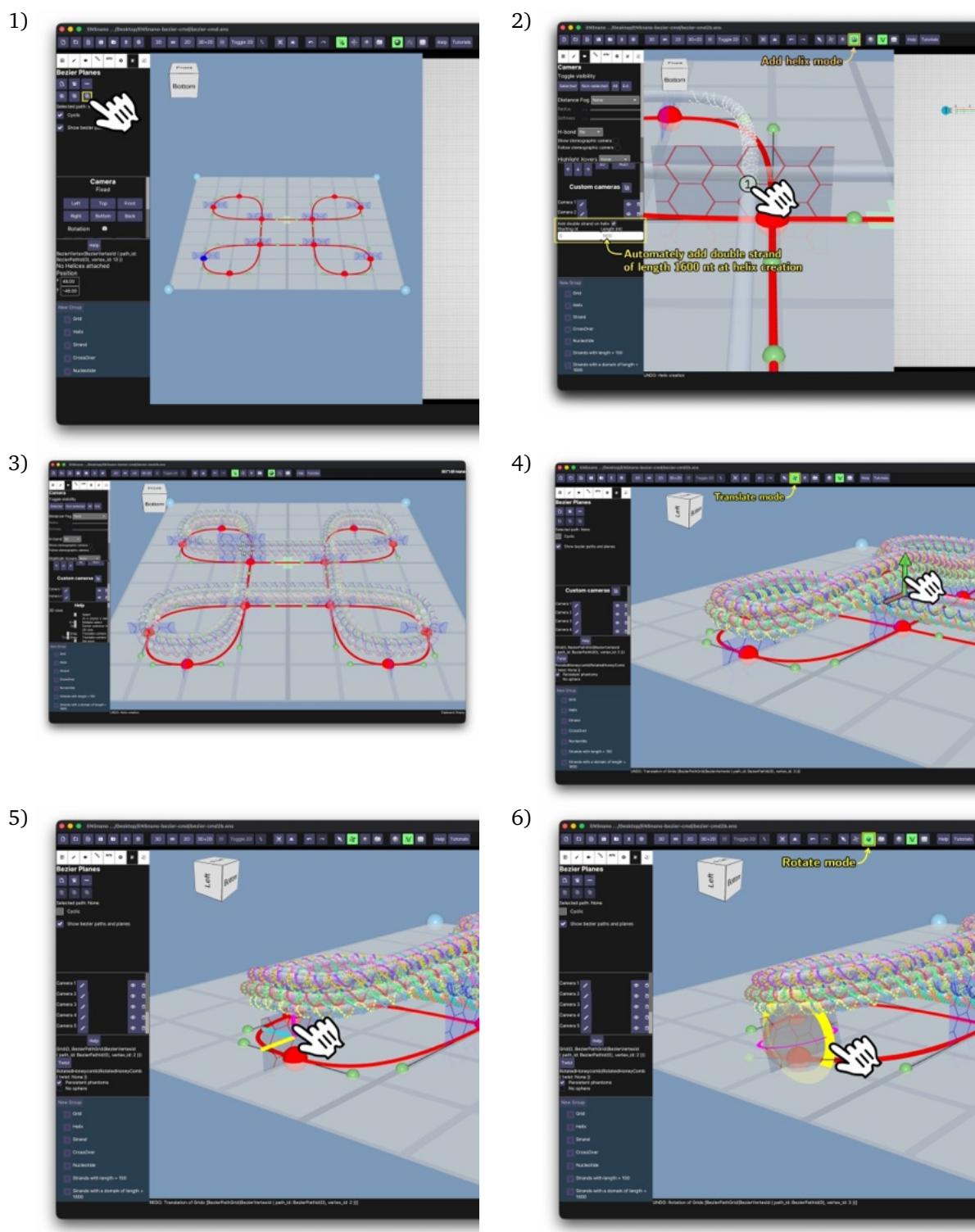


Figure S15: Step-by-step design of the four-loop DNA origami structure in ENSnano (steps 1–6). Once the Bézier path has been defined, (1) attach a grid to the path (here, a rotated honeycomb grid). (2–3) Switch to “Add Helix” mode and populate the grid with helices; double-stranded DNA can be automatically generated on newly created helices using the dedicated option. (4) Switch to “Translate” mode to displace the grids at path intersections and prevent steric clashes. (5) Switch to “Rotate” mode to adjust the orientation of peripheral helices, thereby smoothing their trajectories and improving bundle geometry.

**Scaling and adjustment of curved DNA bundles.** As shown in fig. S16, ENSnano enables *interactive scaling and adjustment of DNA bundles* derived from Bézier paths while *displaying in real time both the total scaffold length and the individual lengths of all constituent helices in nucleotides*. This provides direct feedback on the effect of geometric modifications, allowing the bundle to be accurately scaled to a target size while satisfying strand-length constraints.

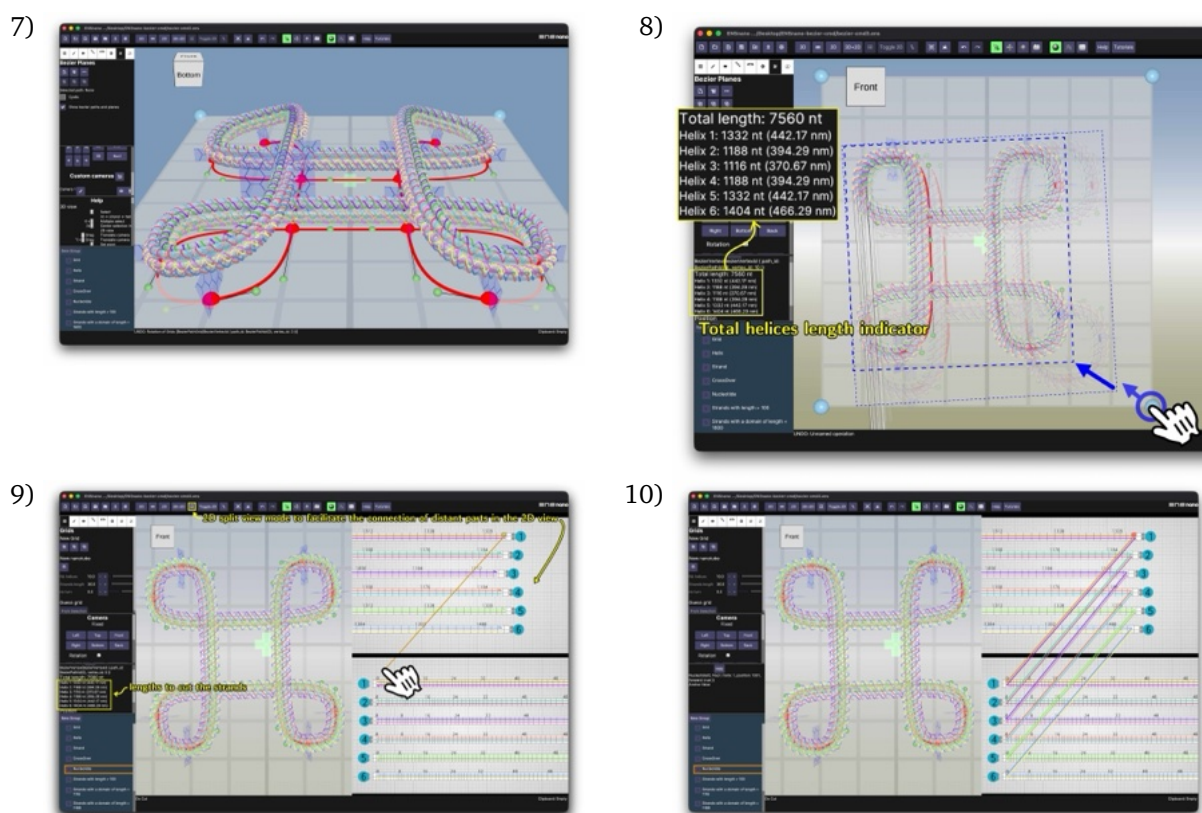


Figure S16: Step-by-step design of the four-loop DNA origami structure in ENSnano (steps 7–10). Once a satisfactory set of helix trajectories has been obtained (7), select a Bézier vertex and use the Bézier-plane handles to scale the design to the target size. To facilitate this process, ENSnano displays in real time the total length of the structure as well as the length, in nucleotides, of each helix attached to the path, enabling precise and intuitive adjustment of the design scale. (9–10) Finally, split the 2D view to simultaneously inspect both ends of the helices, allowing strand closure and length verification.

**Crossover positioning.** ENSnano's adaptive 2D view maps DNA-bundle helices onto straight helices while preserving their cross-sectional organization, ensuring that nucleotides occupying the same position within the bundle remain aligned in the 2D representation (figs. S8 and S9). Furthermore, ENSnano provides a split 2D view that enables the simultaneous visualization of two distinct regions of a design. This makes it straightforward to connect parts of the structure that are spatially adjacent in 3D but separated in the 2D representation. In the four-loop origami, the split view facilitates the placement of crossovers between the upper and lower sections of the bundle at the four junctions where the helices overlap as shown in fig. S17.

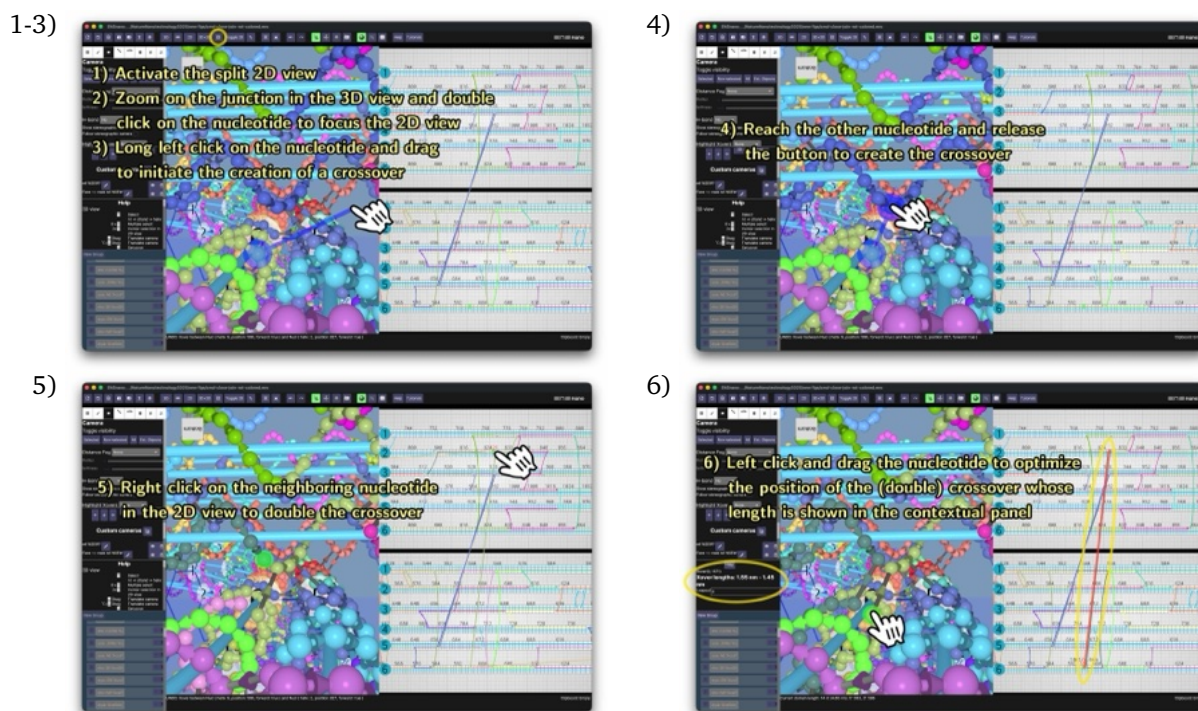


Figure S17: Positioning joining crossovers in the four-loop bundle. (1–3) Switch to the split 2D view and zoom each panel onto one of the two regions to be connected, using double-click navigation on nucleotides and a locally created crossover in the 3D view as a spatial reference. (4–6) Alternate between the 2D and 3D views to construct and fine-tune the double crossovers between the two regions.



## S2.2 Overview of the default joining pattern in the four-loop bundle

In its default design, the  $\mathbb{H}$ -origami contains four sets of staple strands, denoted NW, NE, SW, and SE, that bridge the upper and lower portions of the structure at the four crossover junctions shown in fig. S18.

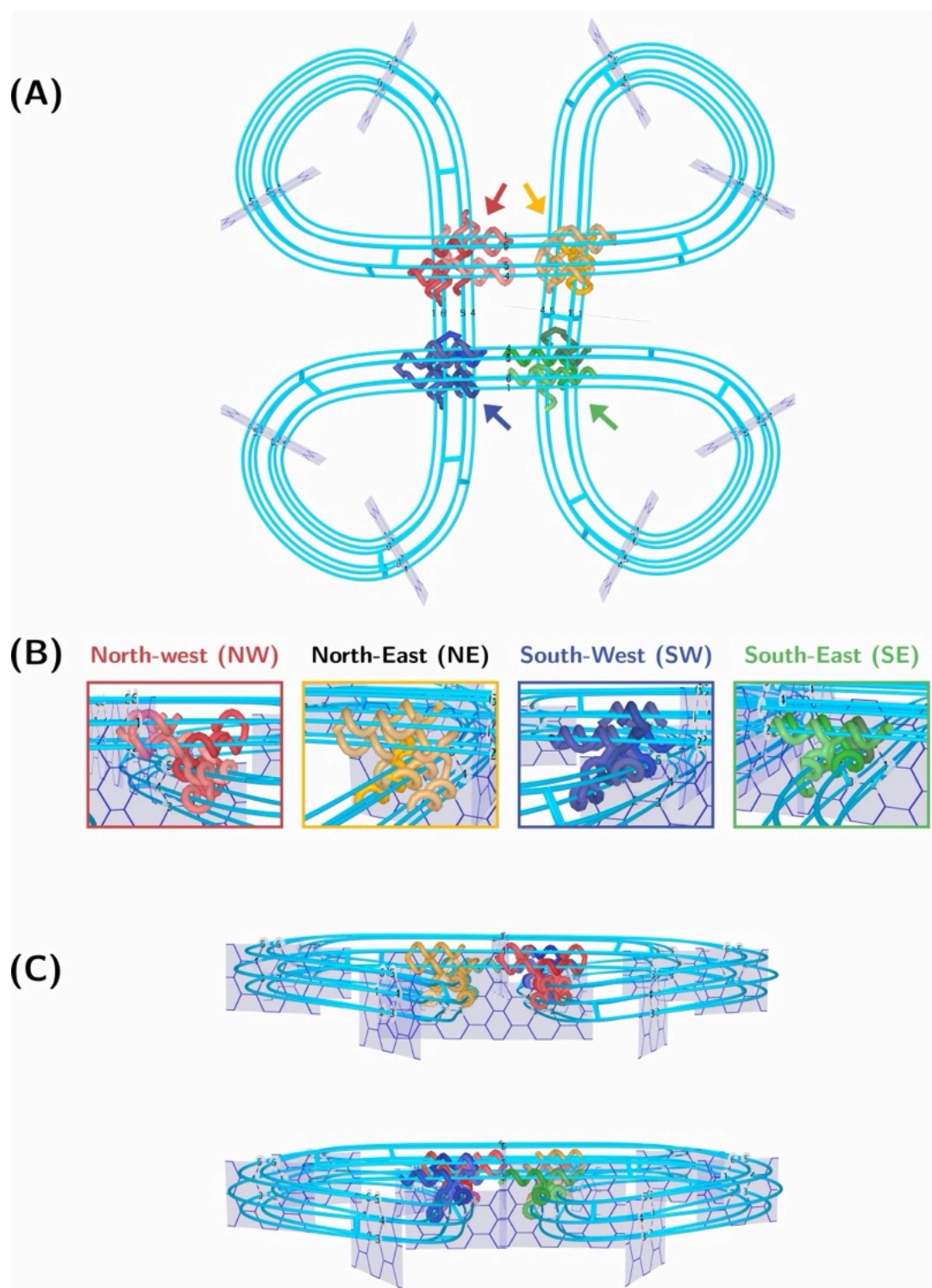


Figure S18: Default joining pattern in the  $\mathbb{H}$ -origami. For clarity, only the scaffold strand (shown in blue, in axis view) and the joining staple strands are displayed. The NW, NE, SW, and SE joining staple sets are coloured red, yellow, blue, and green, respectively. (A) Top view of the structure. (B) Enlarged views of the four joining junctions. (C,D) Side views illustrating the connections between the upper and lower portions of the bundle.

The following figures provide a detailed overview of the  $\Re$ -origami in its default joining configuration, displayed using the three visualization modes available in ENSnano: helix, on-axis, and full-strand representations. In the helix representation, double-stranded DNA helices are rendered as continuous (possibly curved) cylinders. In the on-axis representation, nucleotides are displayed along the helical axes, highlighting the overall crossover pattern while reducing visual complexity. In the full-strand representation, nucleotides are positioned according to the geometric DNA model implemented in ENSnano (described in section S1.3), providing the most accurate depiction of strand geometry and crossover placement.

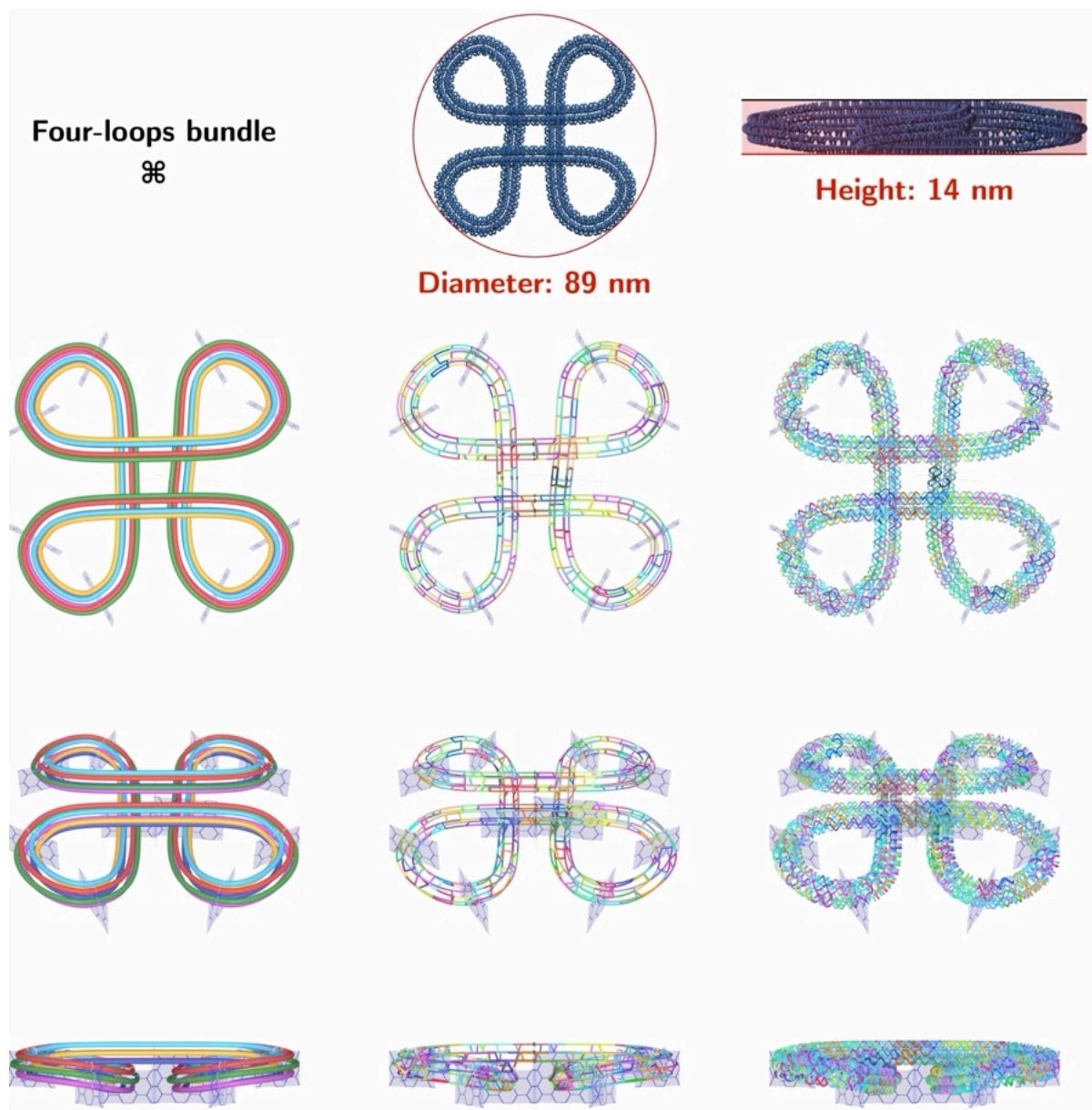


Figure S19: 3D views of the  $\Re$ -origami design. (Top) The structure has a diameter of 89 nm and a height of 14 nm. Rows (top to bottom): top, three-quarter, and side views. Columns (left to right): helix, on-axis and full-strand representations.



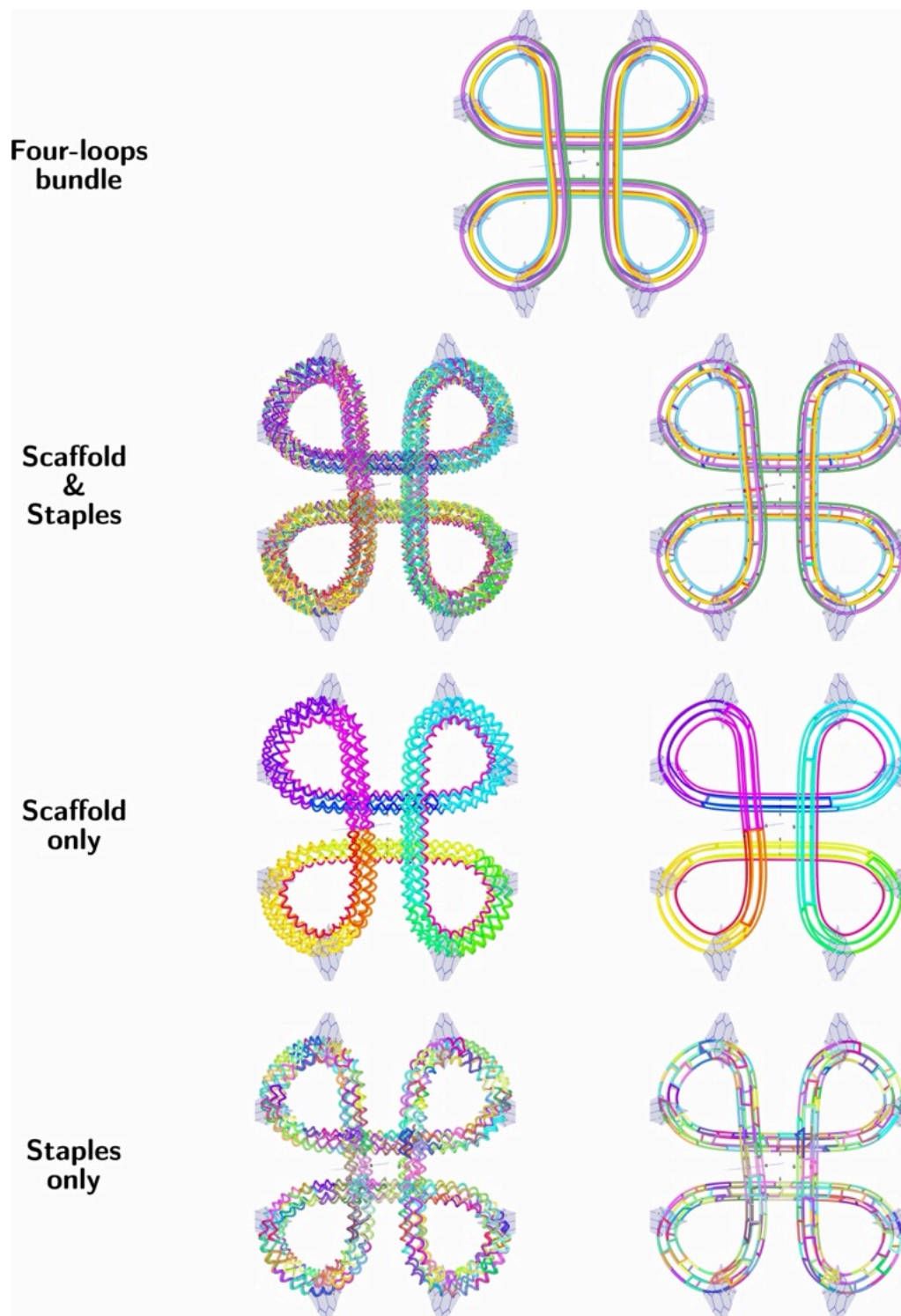


Figure S20: 3D view of the 8-origami design. Full (left) and line (right) representations. Bottom views of the six helices trajectories (top), the scaffold and staples together (middle up), the scaffold alone (middle down) and the staples alone (bottom). Transversal bars on the line representation represents a crossover. The staples shown here are the core staples of the origami, the staples involved in the four junctions (NW, NE, SW, SE) and the staples of the origami in the close join state.

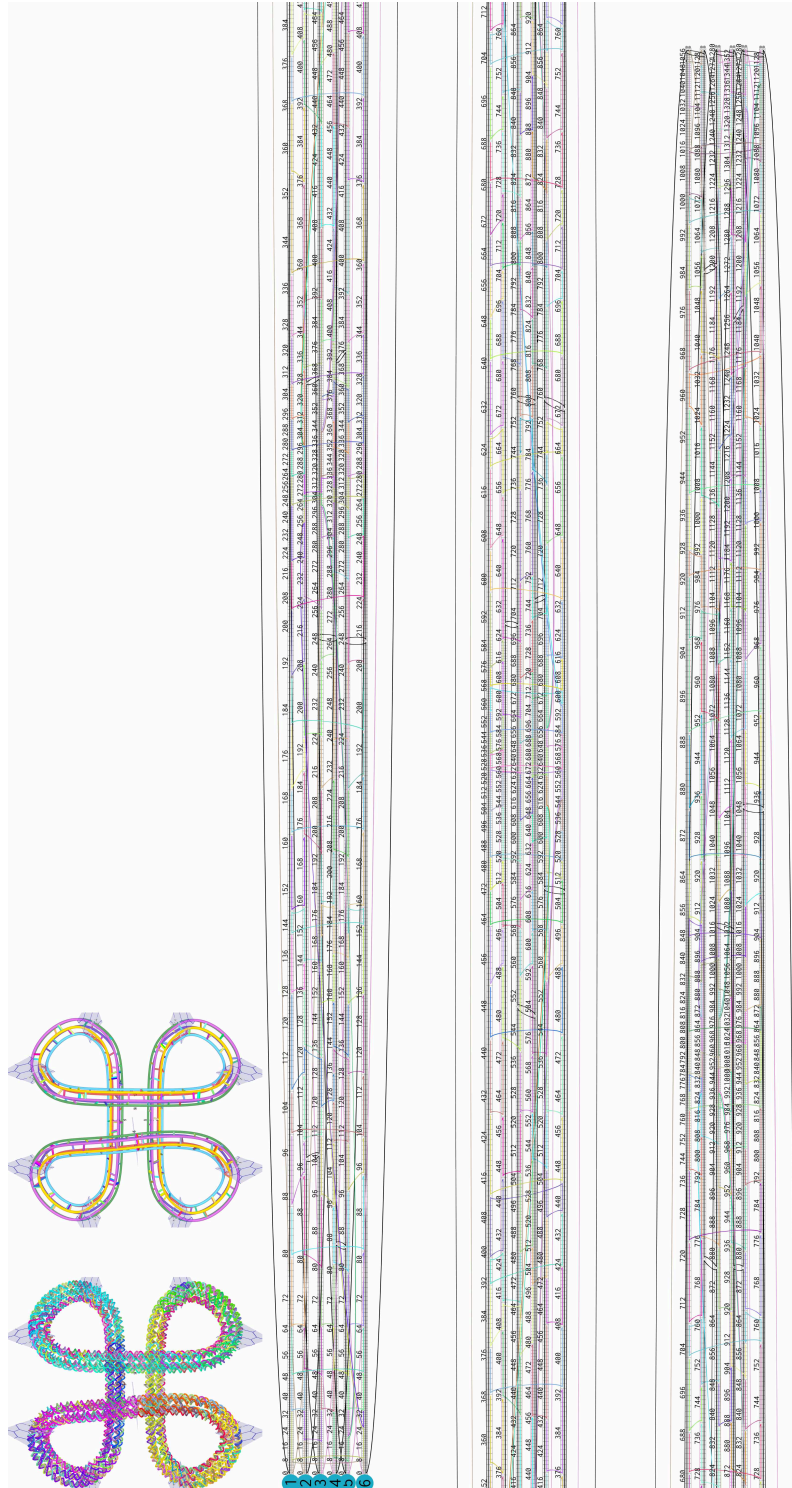
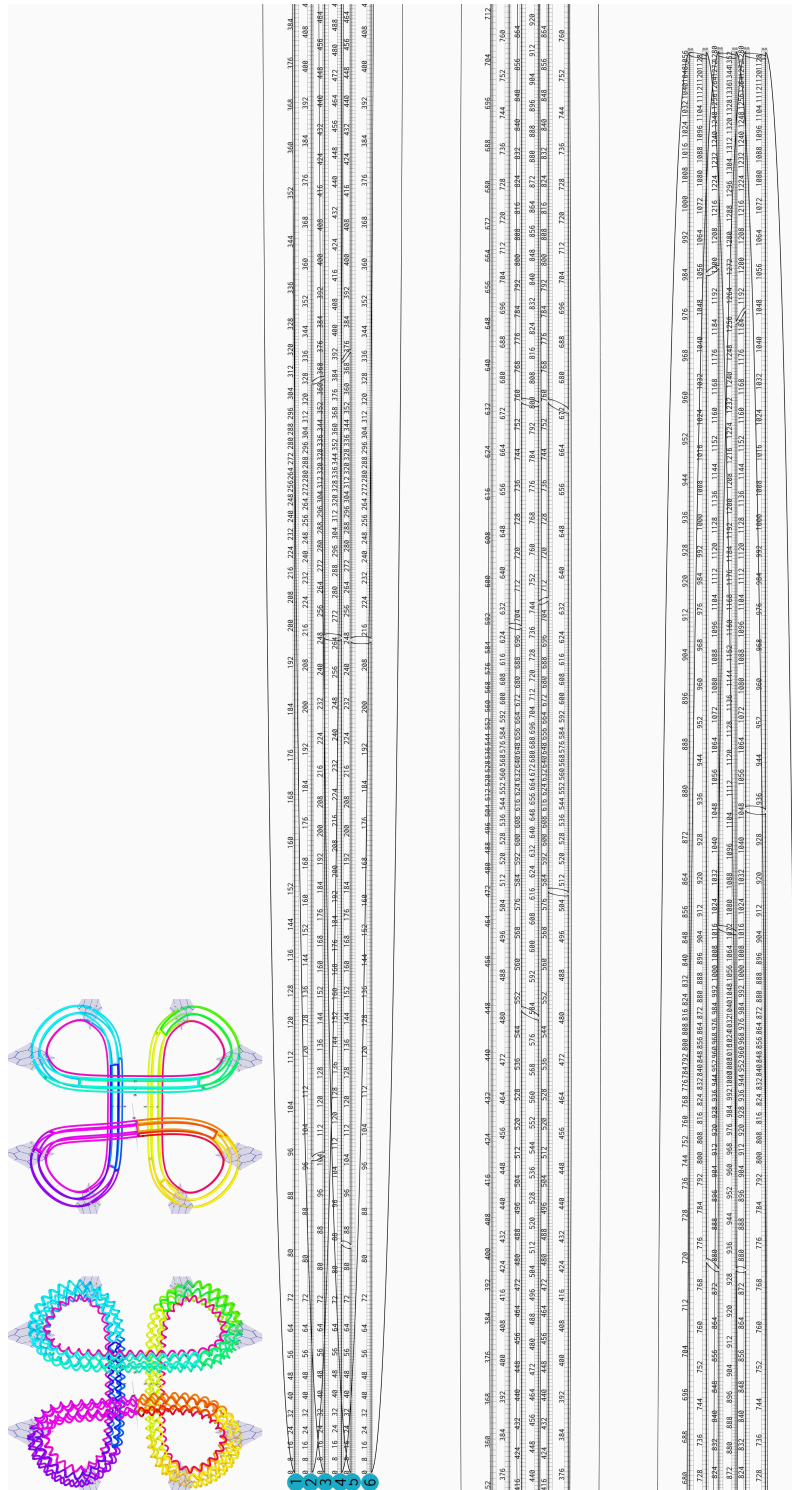


Figure S21: 2D view of the 8-origami design (Scaffold and staples). The staples shown here are the core staples of the origami, the staples involved in the four junctions (NW, NE, SW, SE) and the staples of the origami are in the close state.



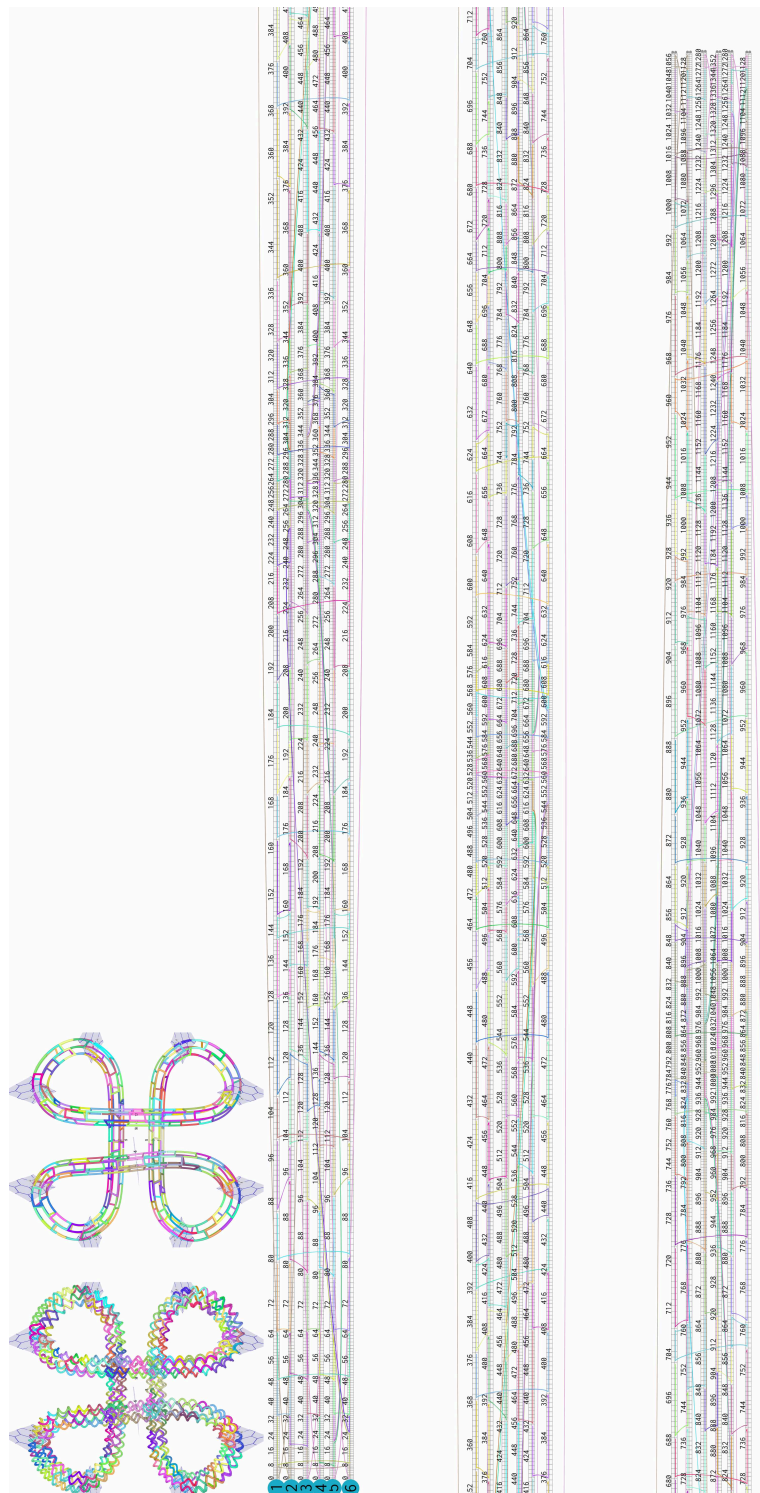


Figure S23: 2D view of the 8-origami design (Staples only). The staples shown here are the core staples of the origami, the staples involved in the four junctions (NW, NE, SW, SE) and the staples of the origami are in the close state.



## S2.3 Scaffold routing and staple geometries

In the  $\mathfrak{K}$ -origami design, we used the classic "leaf-shaped" scaffold consisting of several copies of the same leaf-pattern as illustrated in fig. S24.

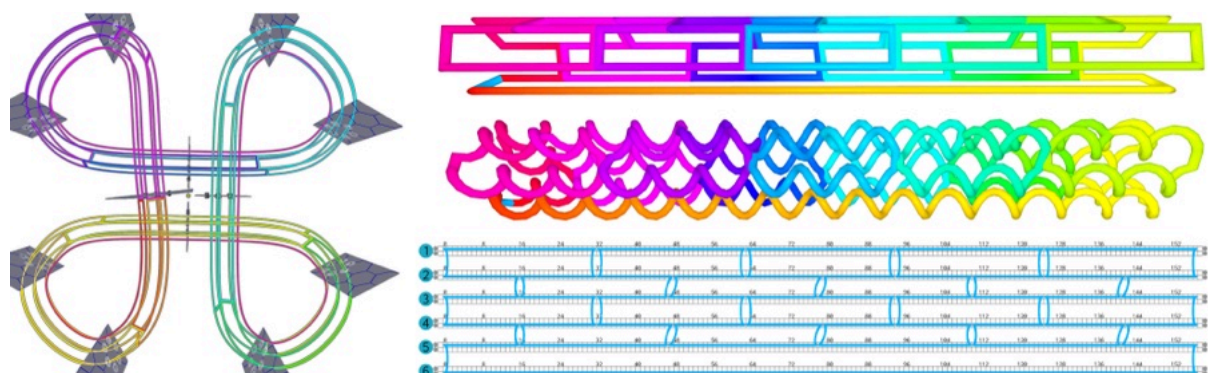


Figure S24: Leaf-shaped scaffold principle used in the  $\mathfrak{K}$ -origami.



In all our designs, we favored the C-shaped staples pattern as illustrated in fig. S25.

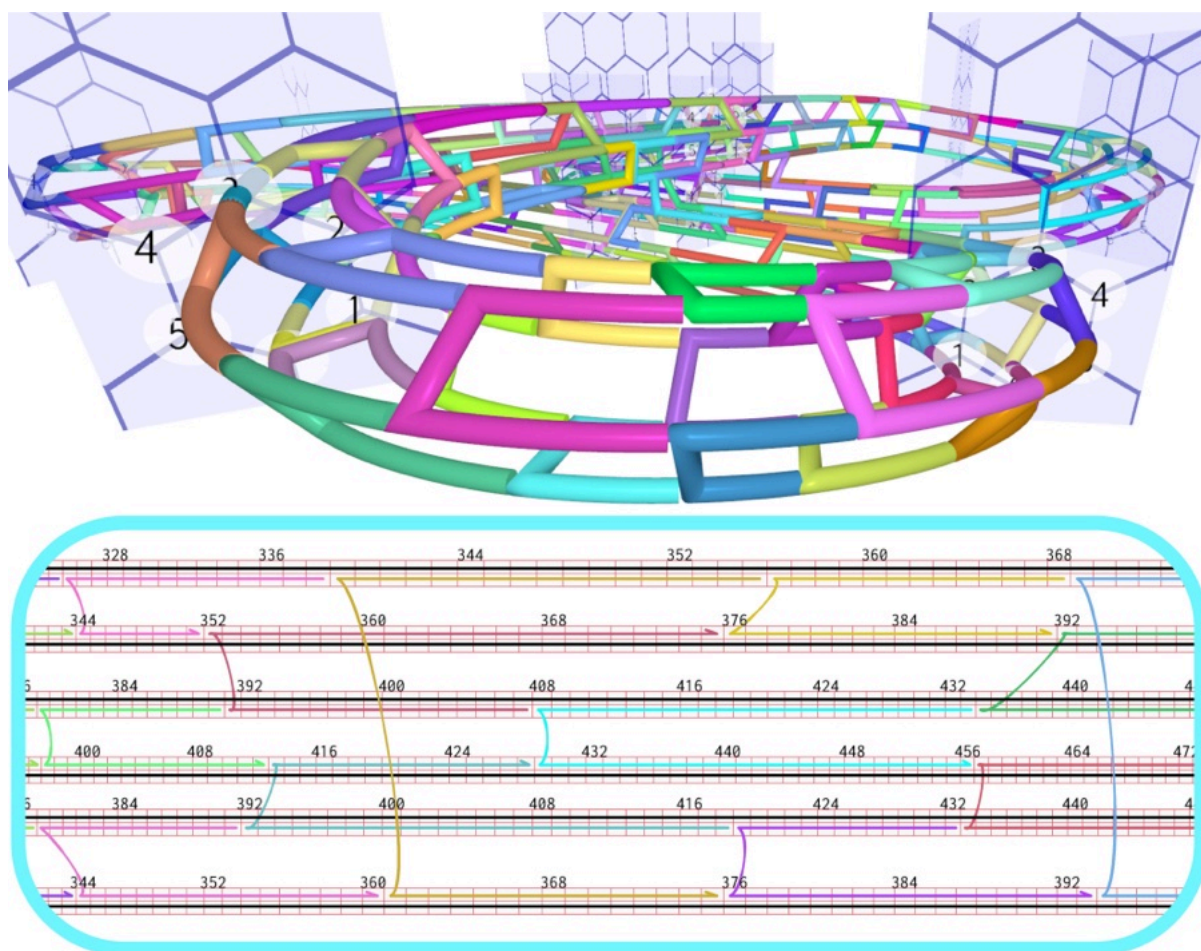


Figure S25: C-shaped staples principle used in every one of our origamis (illustrated here with the  $\mathfrak{K}$ -origami).

## S2.4 Experimental validation of the design of the four-loop bundle

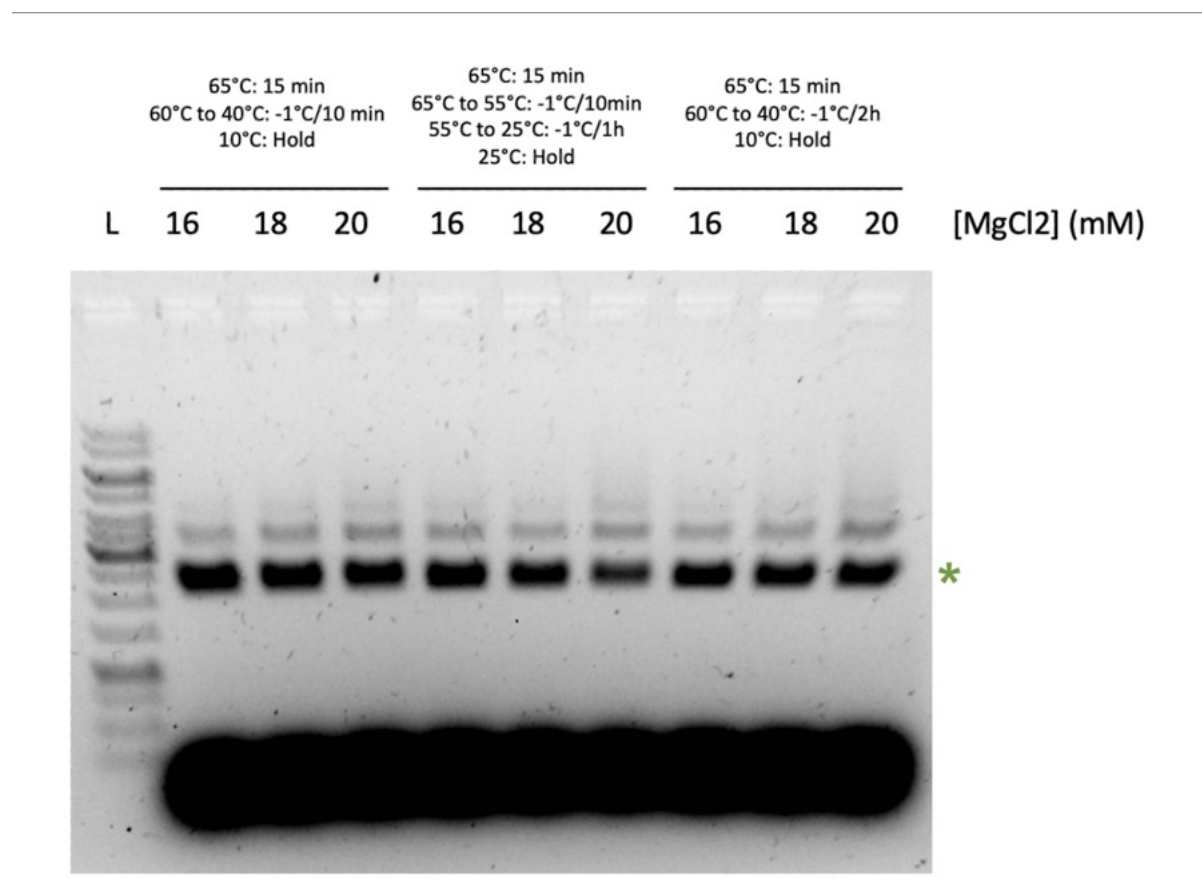


Figure S26: Gel analysis of the influence of MgCl<sub>2</sub> concentration and different folding ramps on folding quality of the ⌘-origami. The foldings were analyzed by agarose gel electrophoresis (1%). L: 1kb DNA ladder. The folding condition of 20 mM MgCl<sub>2</sub> was selected as a compromise between improved folding efficiency, as confirmed by ns-TEM analysis, and increased multimer formation.

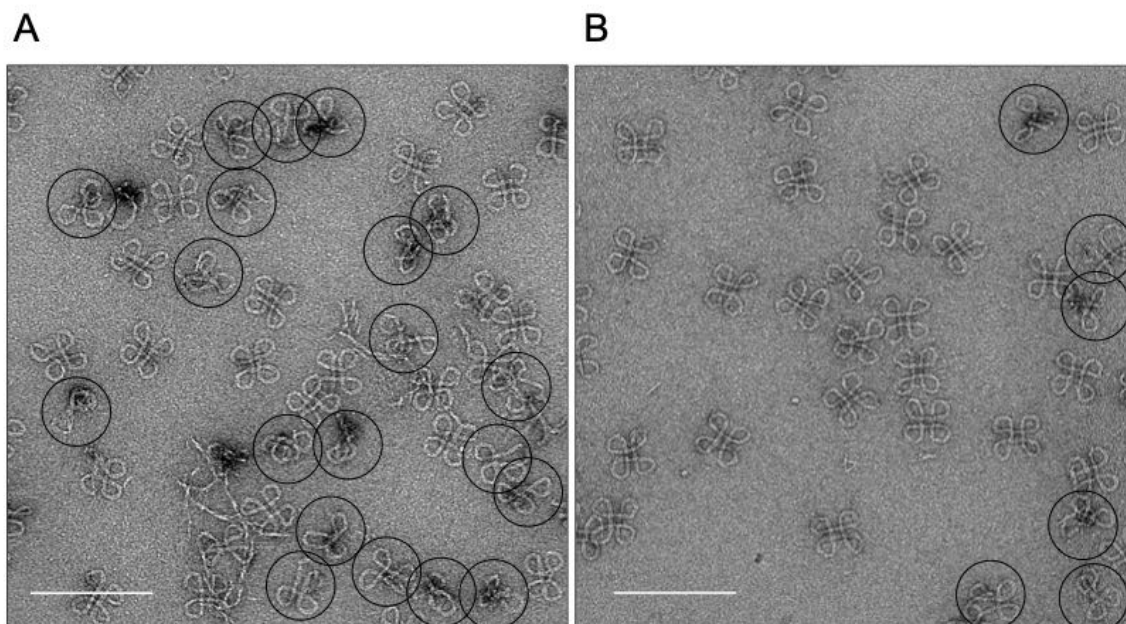


Figure S27: Transmission Electron Microscope (TEM) micrographs of purified ̳-origami folded with 20 mM  $\text{MgCl}_2$  with different folding ramps. A: ̳-origami folded with the following folding ramp: 65°C for 15 minutes, 60°C to 40°C -1°C/10 minutes, 10°C Hold ; B: ̳-origami folded with the following folding ramp: 65°C for 15 minutes, 60°C to 40°C -1°C/2h, 10°C Hold. Particles not folded in the full ̳-origami are circled in black. Scale bar is 200 nm.

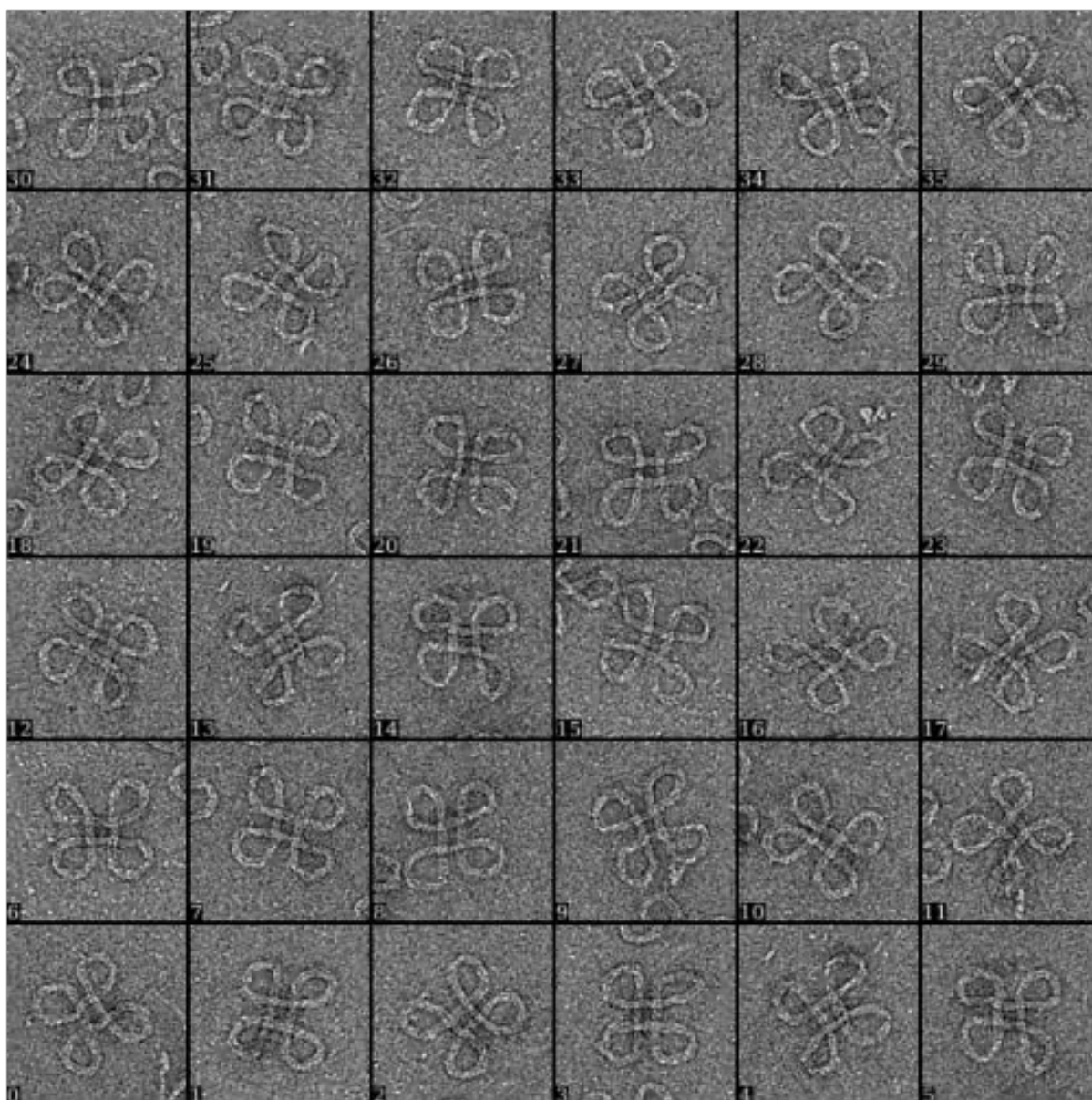


Figure S28: Transmission Electron Microscope (TEM) micrographs of typical particles of the ⌘-origami in optimal folding conditions. Each box is 132 nm x 132 nm.

## S2.5 Variants of the four-loop design

To investigate the effectiveness of the curvature programming induced by the crossover pattern in the loops designed using the ENSnano curved-DNA model, we considered several design variants:

- Three variants of the staples joining the two ends of the scaffold strand: a *closed* variant, an *open* variant in which the two scaffold ends remain capable of stacking, and a *open-T* variant in which each joining staple carries a poly-thymidine extension, separating the scaffold ends and preventing end-to-end stacking.
- An alternative set of staples that does not connect the upper and lower portions of the bundle at the four junctions.

## S2.6 Open and close variants

Figure S29 illustrates the closed and open variants. The open-T variant is obtained by extending the open staples with poly-thymidine overhangs, thereby preventing end-to-end stacking of the scaffold ends.

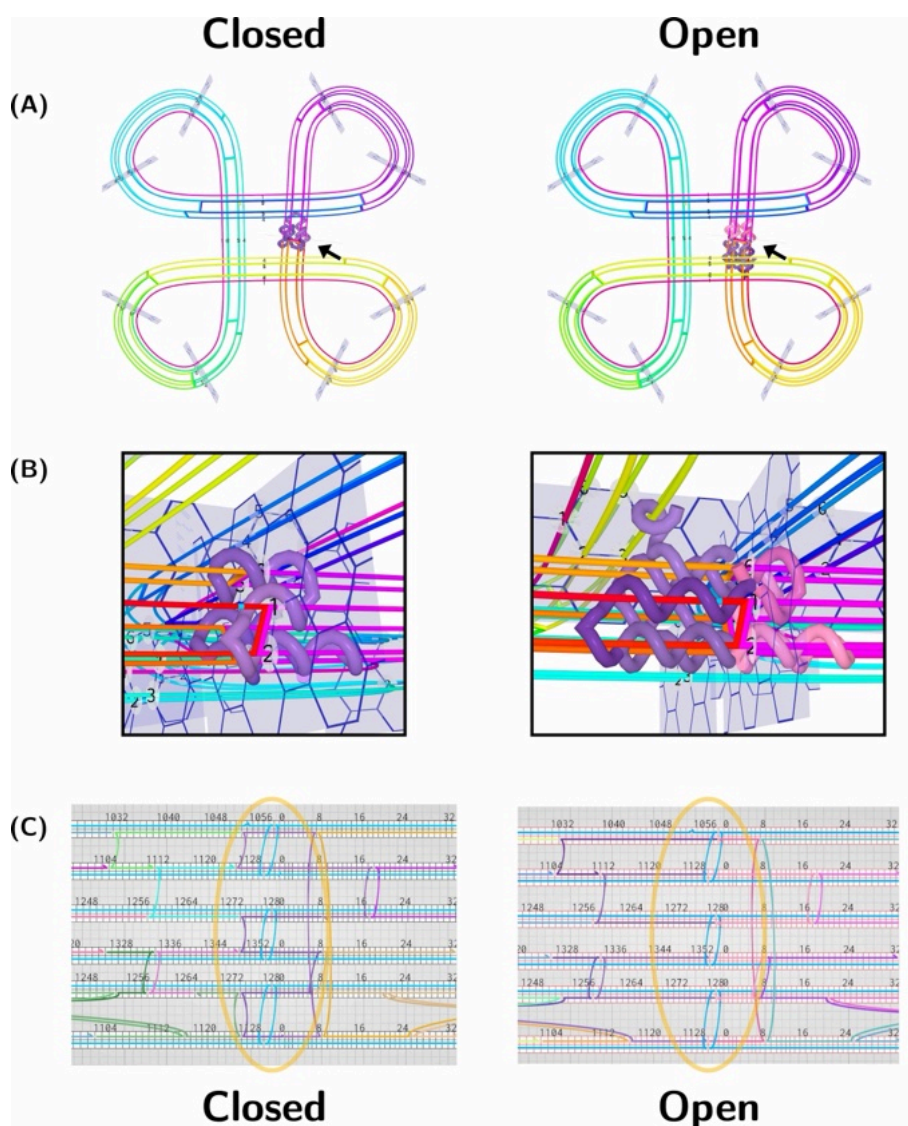


Figure S29: 2D and 3D representations of the closed and open variants of the 8-origami. The closed and open staple designs are shown on the left and right, respectively. (A,B) Top and close-up views of the structure, displaying only the scaffold strand (in axis representation and rainbow coloring) and the corresponding joining staples for clarity. (C) Exact representation of the design in the 2D view. In the open configuration, the joining staples are split precisely at the scaffold discontinuity. In the closed configuration, they bridge the scaffold ends and overlap each side by at least 4–6 base pairs on each helix.



## S2.7 Joined and unjoined junction variants

We designed two alternative staple configurations for each of the four junctions (NW, NE, SW, and SE), shown in fig. S30. Specifically, at each junction:

- In the *join* configuration, two double crossovers connect the upper and lower portions of the bundle.
- In the *no-join* configuration, the bridging staples are severed, leaving the upper and lower portions disconnected at the junction.

Each junction can be independently assigned either the *join* or *no-join* configuration.

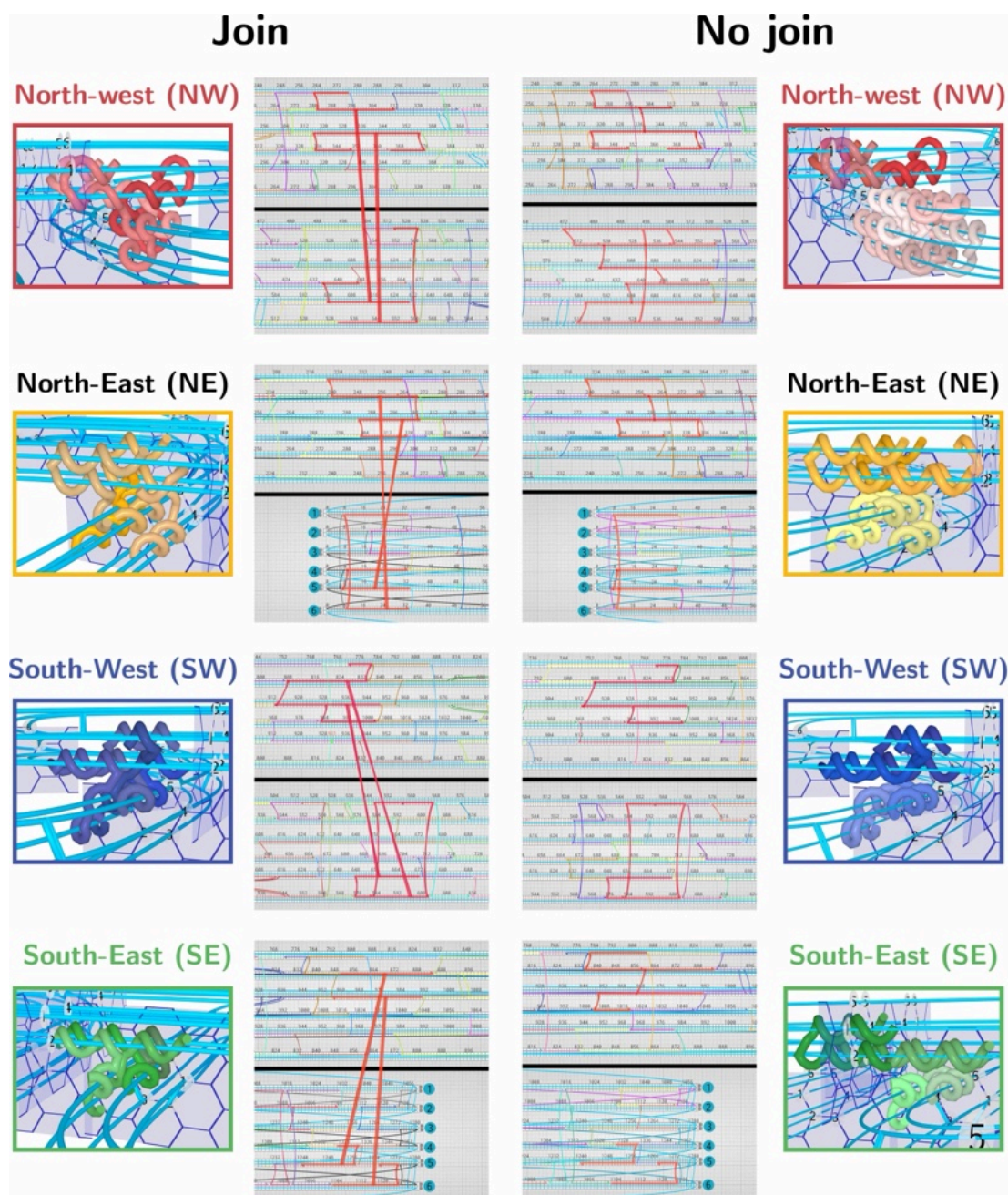


Figure S30: 3D and 2D views of the join and no-join variants at the four junctions of the 3D-origami. For clarity, only the scaffold strand (shown in blue, in axis representation) and the staples involved in the junction are displayed. The join and no-join variants are shown on the left and right, respectively. In the join variant, two double crossovers connect the upper and lower portions of the bundle at the junction. In the no-join variant, these connections are removed, leaving the two portions disconnected.

## S2.8 Study of different conformations of the four-loop bundle depending on the design

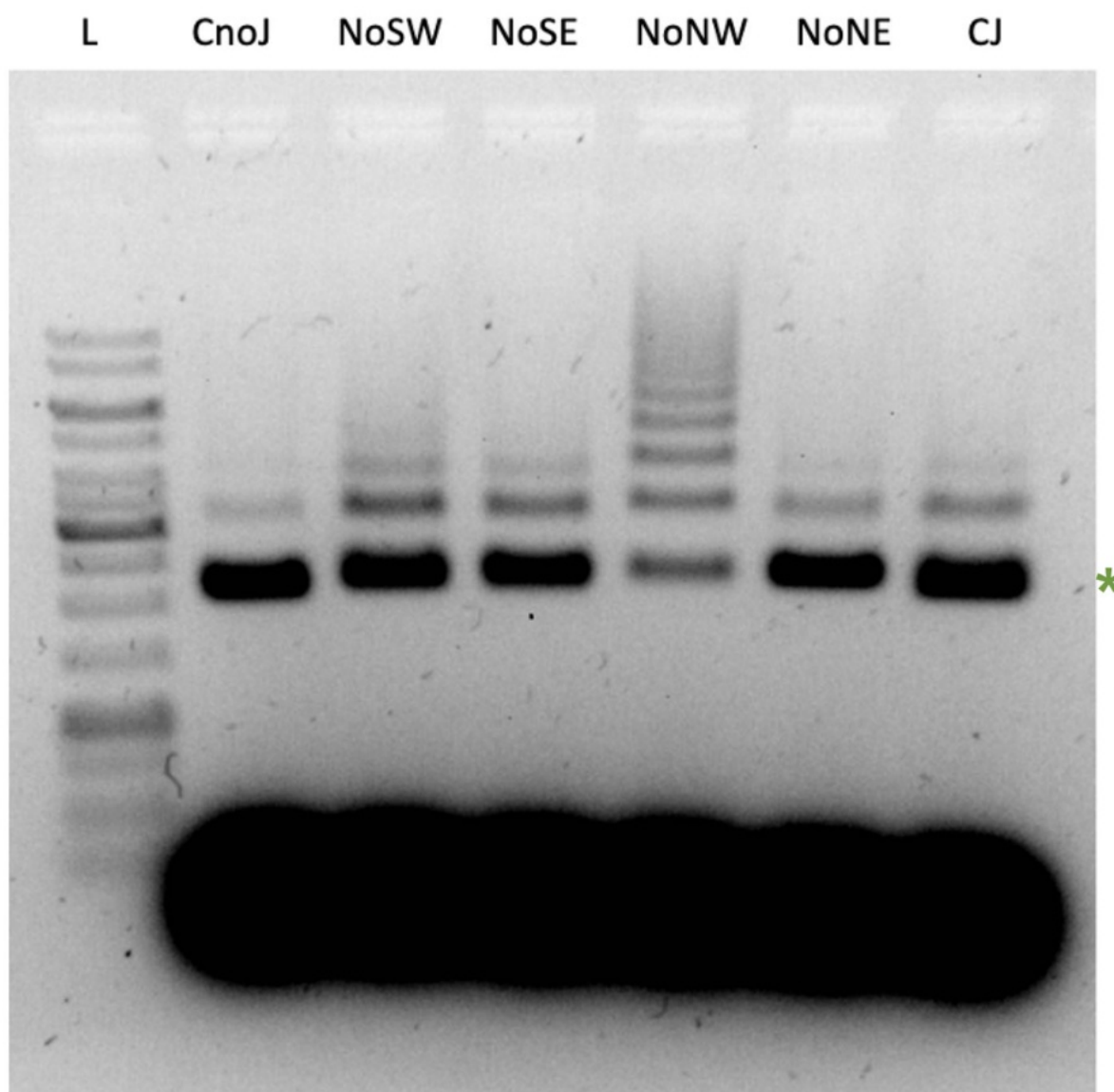


Figure S31: Gel analysis of the influence of the presence of junctions on the folding of the ⌘-origami. The foldings were analyzed by agarose gel electrophoresis (1%). L: 1kb DNA ladder ; CnoJ: ⌘-origami folded in the close state without the junctions NW, NE, SW, SE ; NoSW: ⌘-origami folded in the close state without the junction SW but with the junctions NW, NE, SE ; NoSE: ⌘-origami folded in the close state without the junction SE but with the junctions NW, NE, SW ; NoNW: ⌘-origami folded in the close state without the junction NW but with the junctions SW, NE, SE ; NoNE: ⌘-origami folded in the close state without the junction NE but with the junctions NW, SW, SE ; CJ: ⌘-origami folded in the close state with the junctions SW, NW, NE, SE.

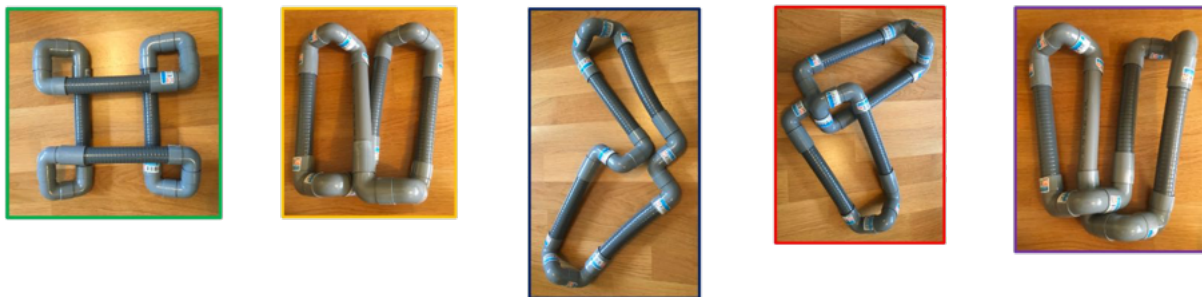


Figure S32: Various structural conformations expected using plumber pipes linked with 3/4-turns corners.

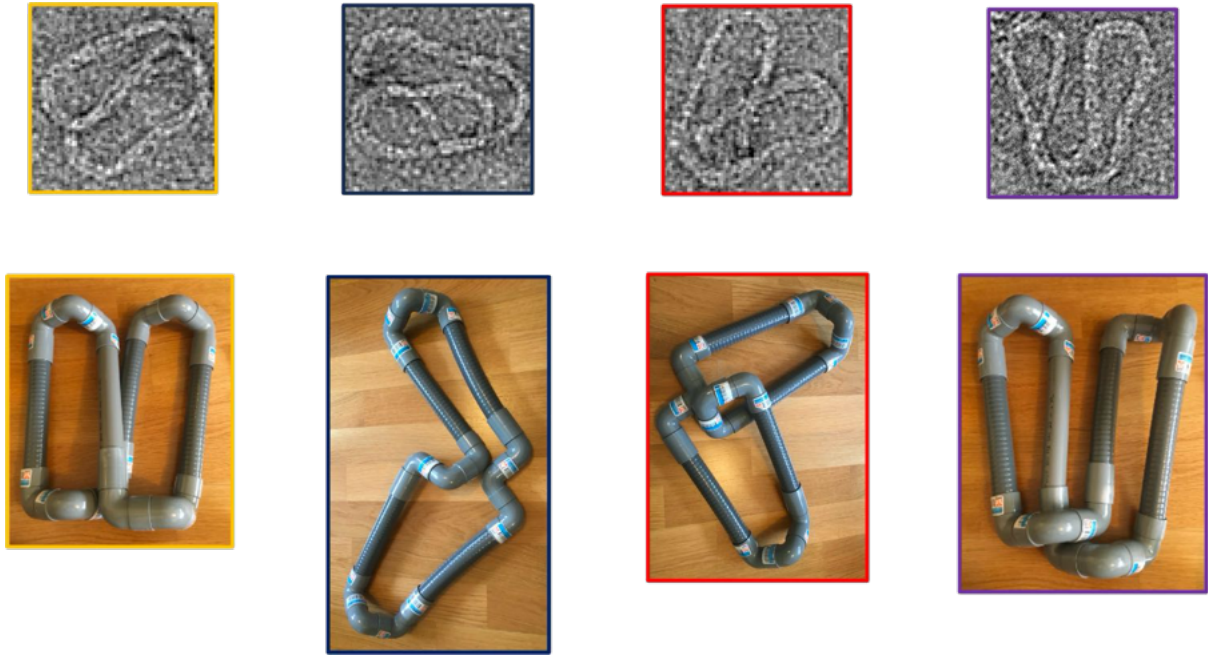


Figure S33: Realization of various shapes observed in Transmission Electron Microscope (TEM) micrographs of purified  $\boxtimes$ -origami using plumber pipes linked with 3/4-turns corners.  $\boxtimes$ -origami folded in the close state, without the junctions NE, SE, NW, SW. Box size of the TEM micrographs = 100 nm.

Interestingly, we observed that some origami folded in the full  $\boxtimes$ -shape even though only one of the four junctions was present, with a better yield when using the staples involved in the NW junction alone than with the other junctions (figs. S34 and S35).

Fully understanding the role of the northwest junction would require a dedicated investigation that falls beyond the scope of the present work. Nevertheless, we can propose two possible explanations. First, the northwest junction is located in the corner opposite the opening of the four-loop bundle (fig. S18) and is therefore likely to occupy a more central position in the open intermediate, potentially making it a more effective nucleation point for the closure process. Second, compared with the southwest junction located at the opposite corner, the northwest junction is structurally more robust because the strands participating in the southwest junction contain shorter binding domains in some of the regions that must assemble together. These hypotheses are consistent with our observations.



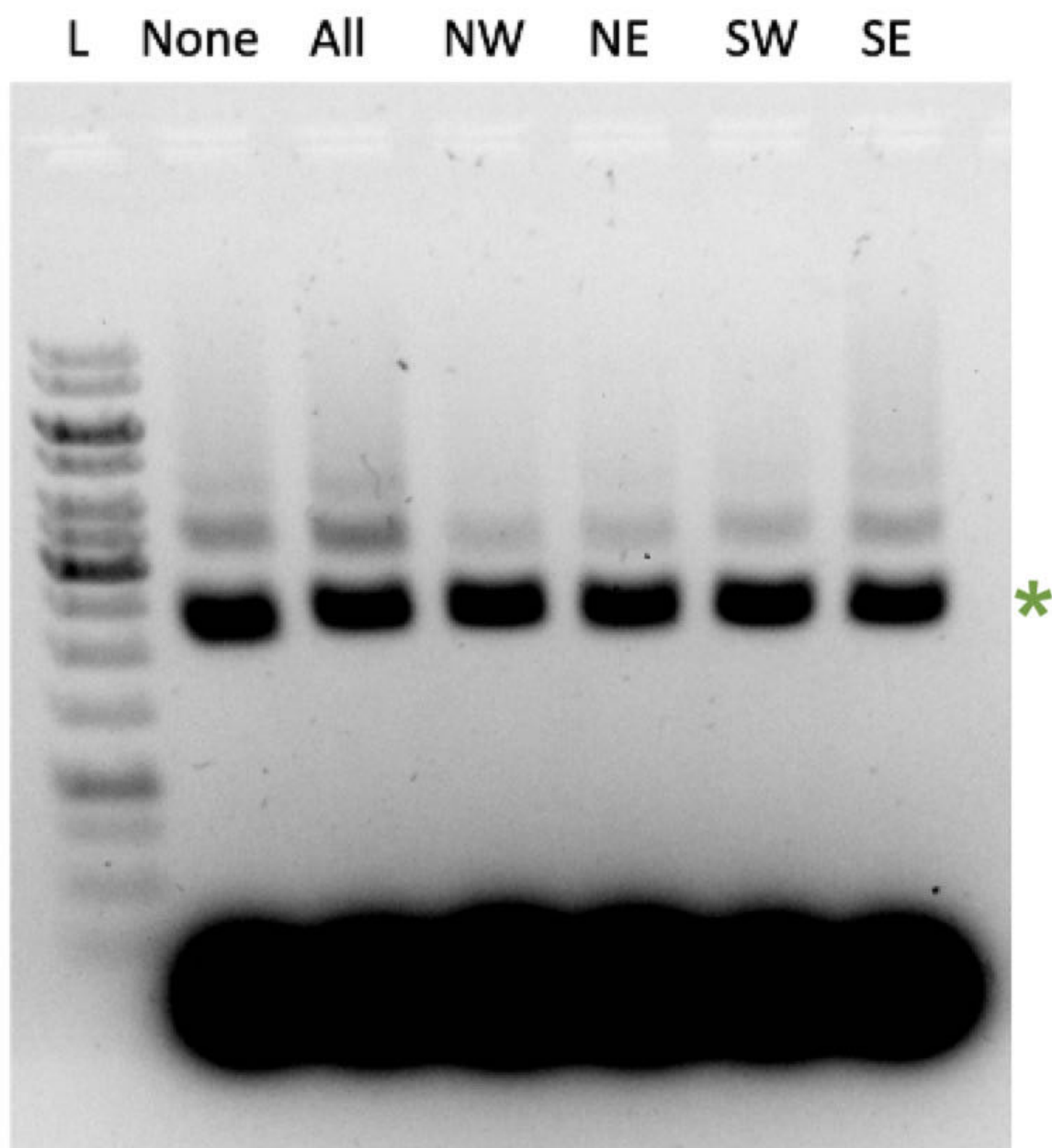


Figure S34: Gel analysis of the influence of the presence of junctions on the folding of the ⌘-origami. The foldings were analyzed by agarose gel electrophoresis (1%). L: 1kb DNA ladder ; None: ⌘-origami folded in the close state without the junctions NW, NE, SW, SE ; All: ⌘-origami folded in the close state with the junctions SW, NW, NE, SE ; NW: ⌘-origami folded in the close state with the junction NW and without the junctions SW, NE, SE ; NE: ⌘-origami folded in the close state with the junction NE and without the junctions SW, NW, SE ; SW: ⌘-origami folded in the close state with the junction SW and without the junctions NW, NE, SE ; SE: ⌘-origami folded in the close state with the junction SE and without the junctions SW, NE, NW.



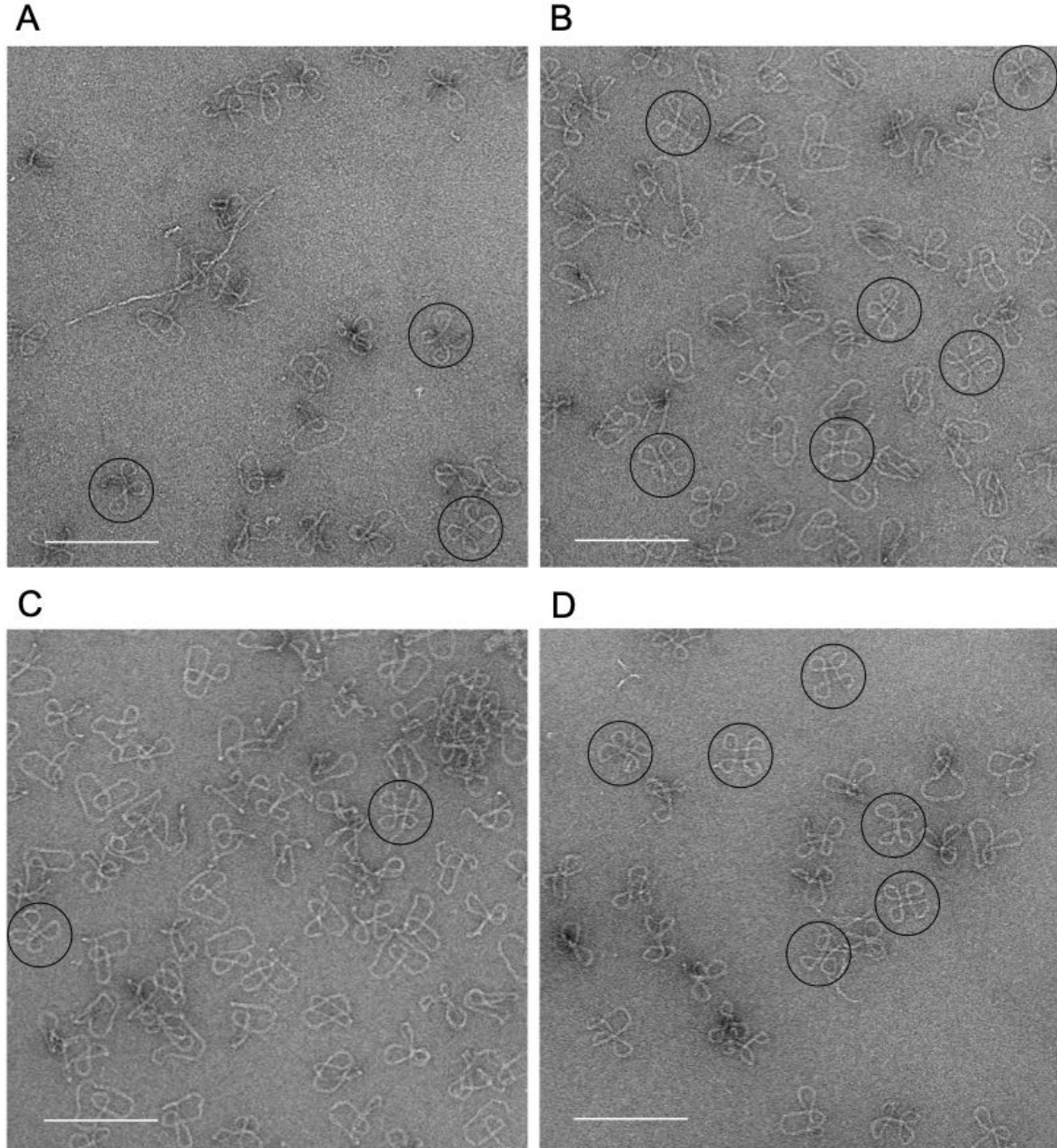


Figure S35: Transmission Electron Microscope (TEM) micrographs of purified  $\Re$ -origami folded in the close state with only one junction out of four. A:  $\Re$ -origami folded with the junction NE and without the junctions SE, NW, SW ; B:  $\Re$ -origami folded with the junction SE and without the junctions NE, NW, SW ; C:  $\Re$ -origami folded with the junction SW and without the junctions SE, NW, NE ; D:  $\Re$ -origami folded with the junction NW and without the junctions SE, NE, SW. Particles folded in the full  $\Re$ -origami are circled in black. Scale bar is 200 nm.

In the same way, we observed on agarose gel electrophoresis as well as with negative-stain transmission electron microscopy that the proportion of particles folded in the full  $\Re$ -origami with the SE, SW and NE but not the NW junctions is less than when the origami is folded using any other triplet of junctions (figs. S31 and S36). These results together suggest that the NW junction staples stabilize the curved-shape better than the other junctions and is enough to stabilize the curvature in the full  $\Re$ -shape.

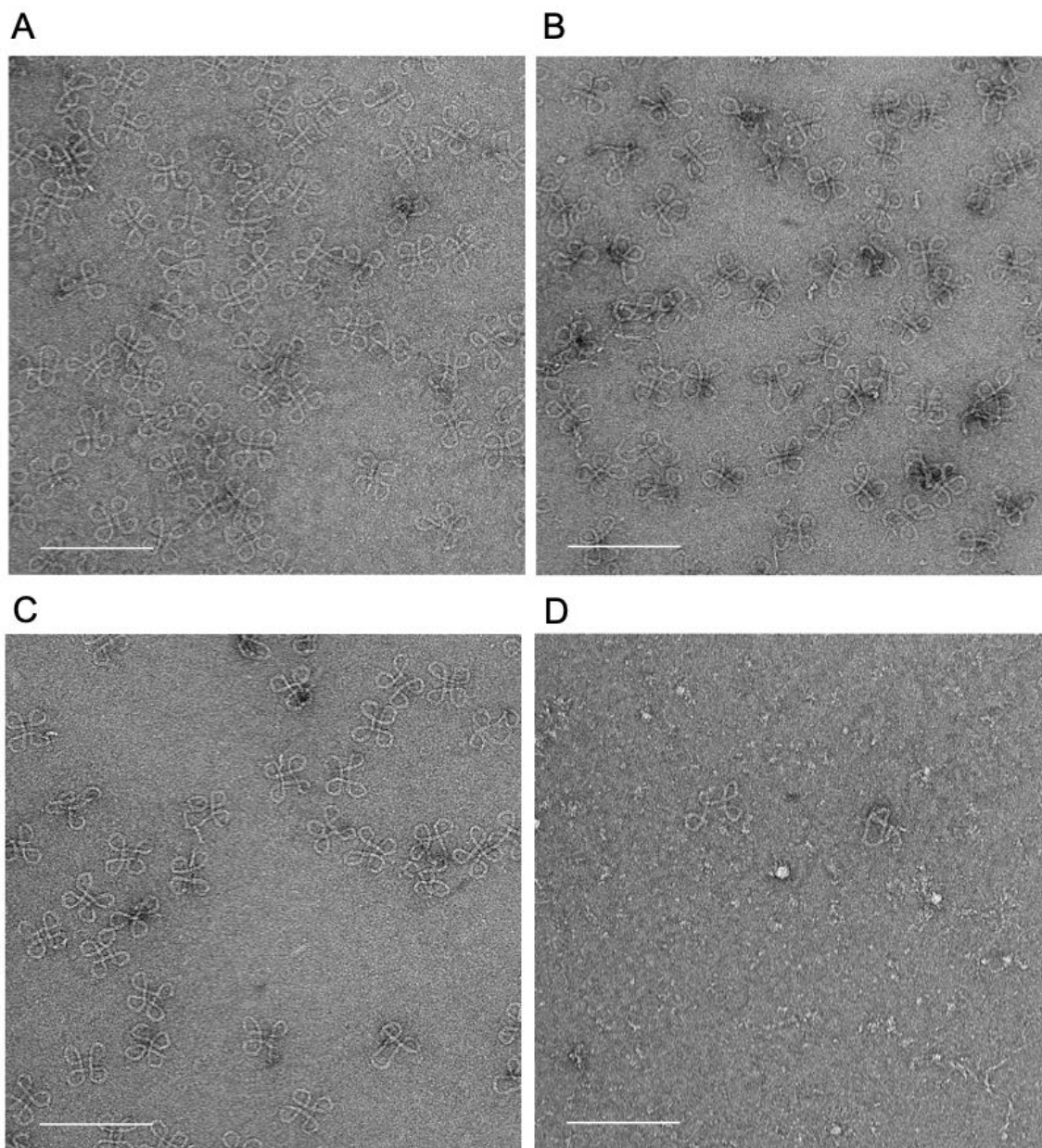


Figure S36: Transmission Electron Microscope (TEM) micrographs of purified  $\boxtimes$ -origami folded in the close state with three junctions out of four. A:  $\boxtimes$ -origami folded without the junction NE and with the junctions SE, NW, SW ; B:  $\boxtimes$ -origami folded without the junction SE and with the junctions NE, NW, SW ; C:  $\boxtimes$ -origami folded without the junction SW and with the junctions SE, NW, NE ; D:  $\boxtimes$ -origami folded without the junction NW and with the junctions SE, NE, SW. Scale bar is 200 nm.

1207 Additionally, when trying to fold the ⌘-origami with only two junctions out of four, we observe that  
 1208 the ⌘-origami can reach its full ⌘-shape, with a better yield when using opposite junctions (e.g. NW  
 1209 and SE or NE and SW) rather than adjacent ones (e.g. NW and NE or NW and SW) (figs. S37 and S38).  
 1210 This can be logically explained by the fact that opposite junctions are in contact with the four sides while  
 1211 adjacent ones are only in contact with three.

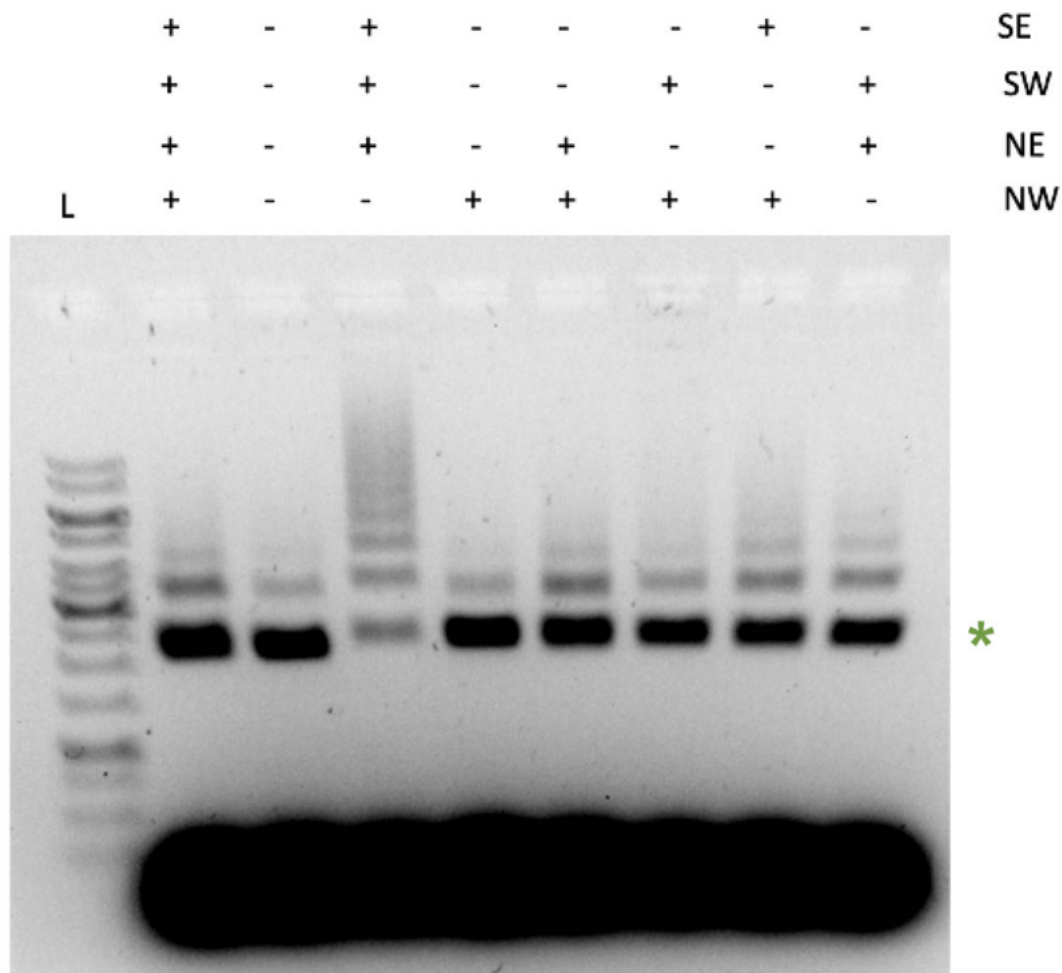


Figure S37: Gel analysis of the influence of the presence of junctions on the folding of the ⌘-origami. All the ⌘-origami were folded in the close state. The foldings were analyzed by agarose gel electrophoresis (1%). L: 1kb DNA ladder.

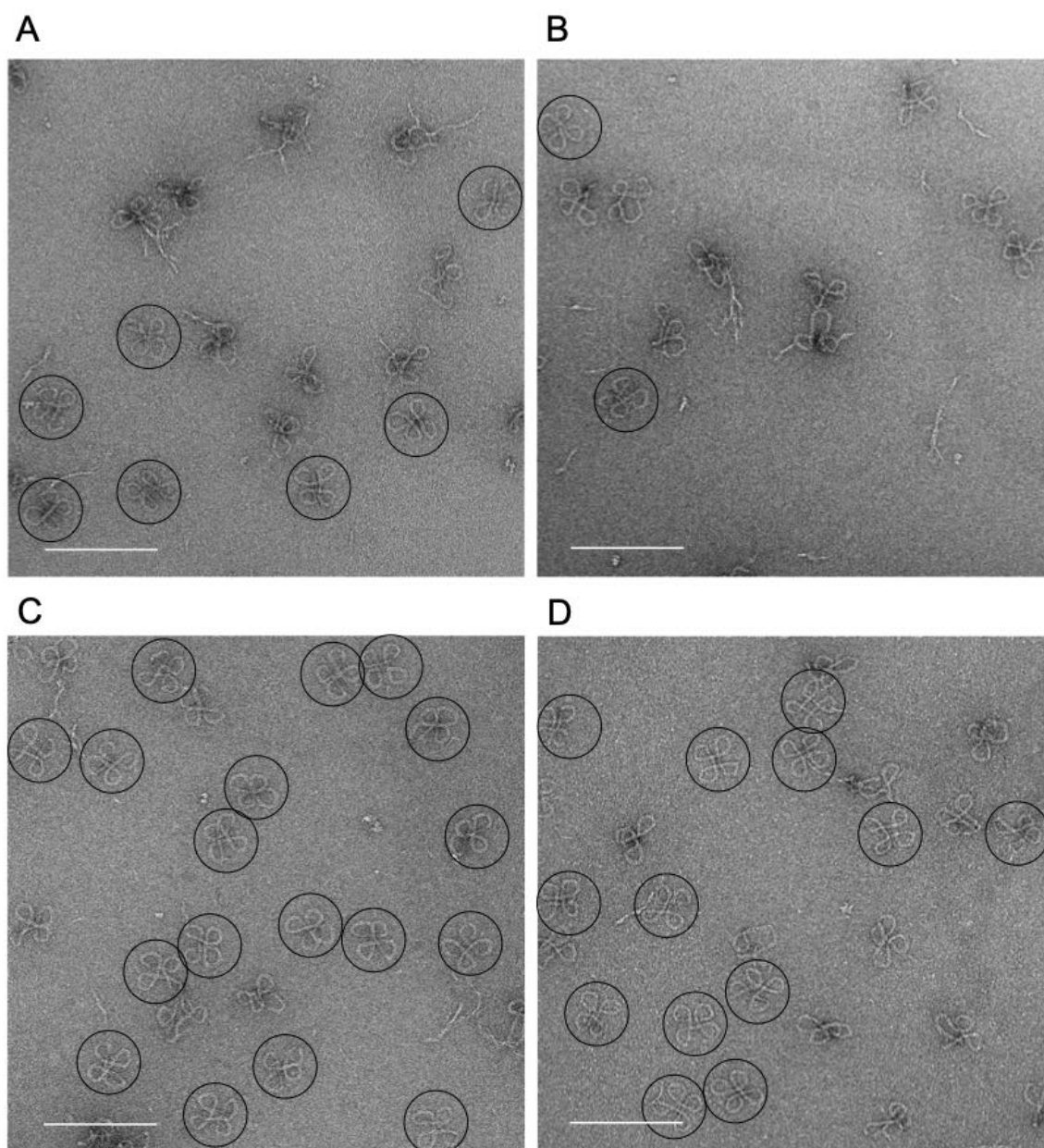


Figure S38: Transmission Electron Microscope (TEM) micrographs of purified 3D-printed origami folded in the close state with two junctions out of four. A and B displays 3D-printed origami with two adjacent junctions whereas C and D displays 3D-printed origami with two opposite junctions. A: 3D-printed origami folded with the junction NW and SW and without the junctions NE and SE ; B: 3D-printed origami folded with the junction NW and NE and without the junctions SW and SE ; C: 3D-printed origami folded with the junction NE and SW and without the junctions NW and SE ; D: 3D-printed origami folded with the junction NW and SE and without the junctions NE and SW. Particles folded in the full 3D-printed origami are circled in black. Scale bar is 200 nm.

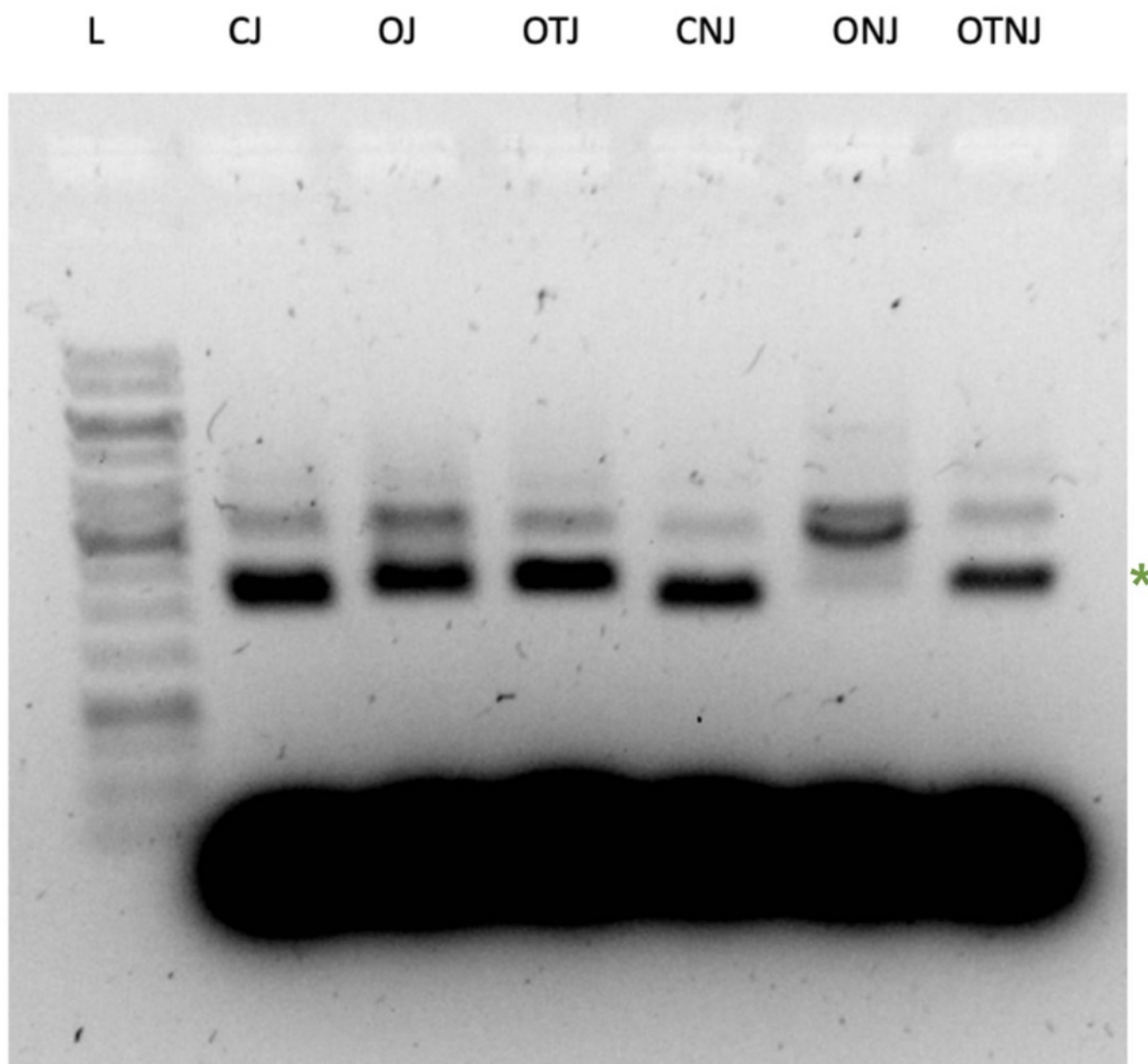


Figure S39: Gel analysis of the influence of the presence of junctions and open, open-T or close state on the folding quality of the ⌘-origami. The foldings were analyzed by agarose gel electrophoresis (1%). L: 1kb DNA ladder. CJ: ⌘-origami folded in the close state with the four junctions: NW, NE, SW, SE ; OJ: ⌘-origami folded in the open state with the four junctions: NW, NE, SW, SE ; OTJ: ⌘-origami folded in the open-T state with the four junctions: NW, NE, SW, SE ; CNJ: ⌘-origami folded in the close state without any of the four junctions: NW, NE, SW, SE ; ONJ: ⌘-origami folded in the open state without any of the four junctions: NW, NE, SW, SE ; OTNJ: ⌘-origami folded in the open-T state without any of the four junctions: NW, NE, SW, SE.



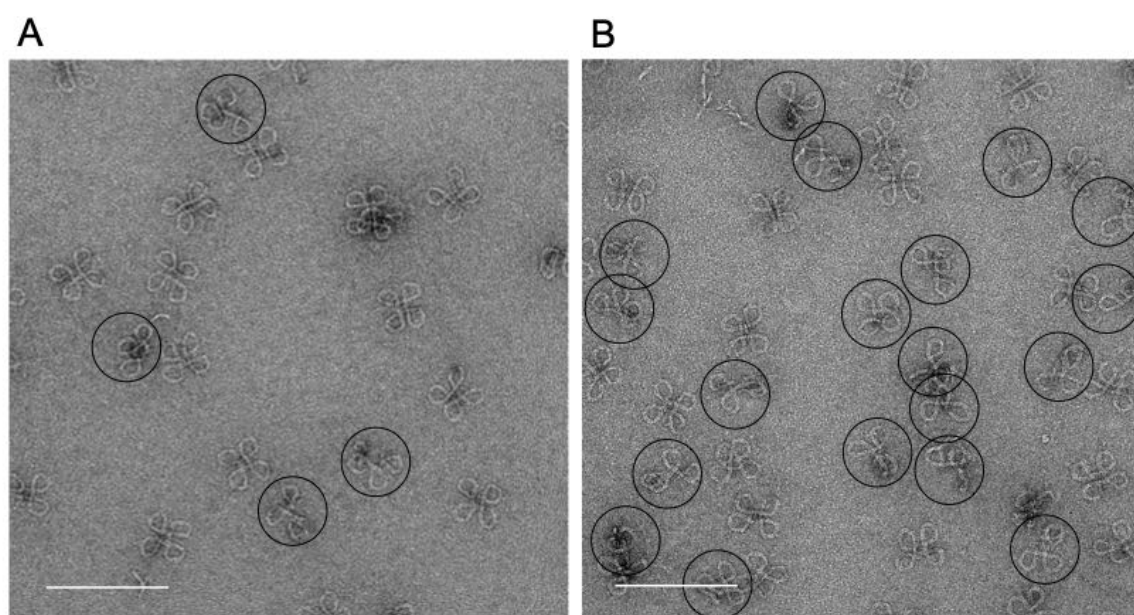


Figure S40: Transmission Electron Microscope (TEM) micrographs of purified  $\boxtimes$ -origami folded in different states with the four junctions NW, NE, SW and SE. A:  $\boxtimes$ -origami folded in the open state ; B:  $\boxtimes$ -origami folded in the open-T state. Particles not folded in the full  $\boxtimes$ -origami are circled in black. Scale bar is 200 nm.

**Open-T no-Junction configuration.** Figure S41 presents the 1% agarose gel electrophoresis analysis and representative TEM micrographs of the  $\Re$ -origami in its least constrained configuration, namely the open-T/no-join variant. The gel exhibits a well-defined band, indicating discrete folding of the origami. The TEM micrographs reveal that the open-T/no-join  $\Re$ -origami adopts conformations consistent with those observed for the closed/no-join variant, with the expected increase in structural flexibility. This confirms that the crossover pattern derived from the three-dimensional nucleotide embedding computed by ENSnano accurately encodes the target bending of the  $\Re$ bundle, despite its minimal architecture of only six parallel DNA helices arranged on a honeycomb lattice.

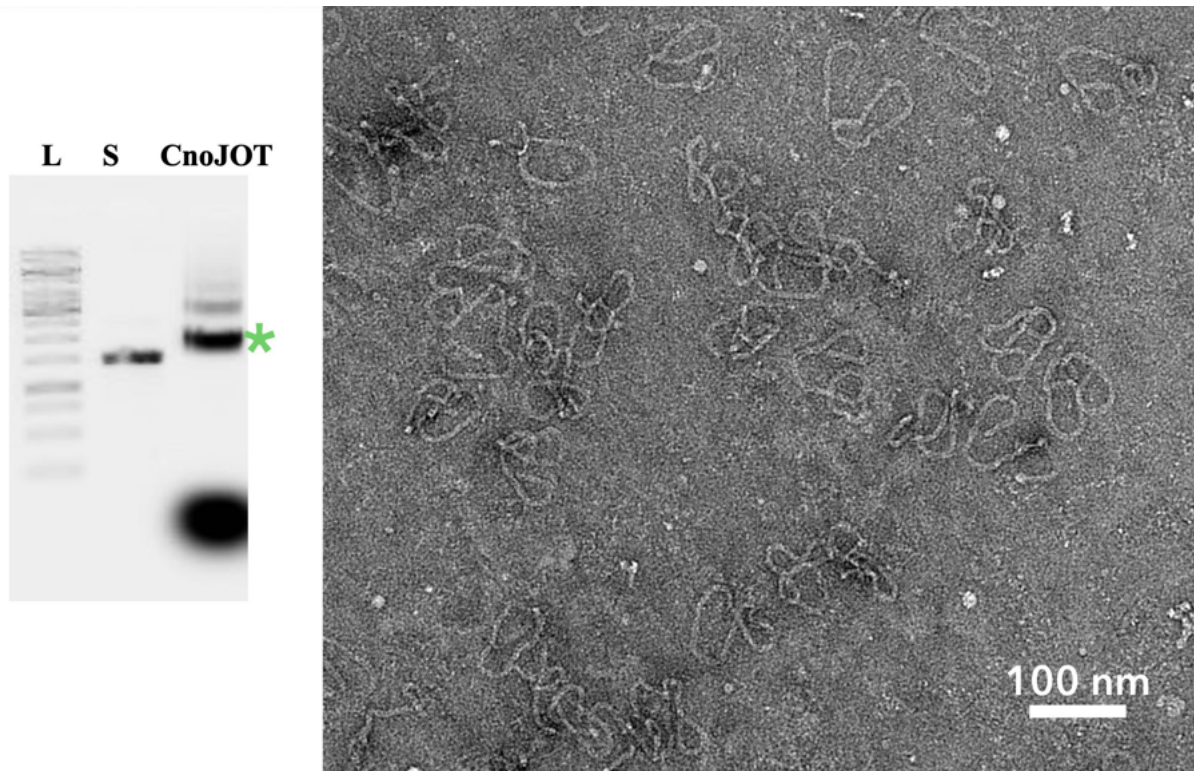


Figure S41: Gel analysis of the  $\Re$ -origami folded in the open-T no-Junction state. Folding products were analyzed by 1% agarose gel electrophoresis. L: 1 kb DNA ladder; S: 20 nM p7249 scaffold; CnoJOT:  $\Re$ -origami folded in the open-T configuration with all four junctions (NW, NE, SW, and SE) in the no-join state. Poly-thymidine extensions were added to the joining staples at both ends of the open bundle to prevent end-to-end aggregation. Representative negative-stain TEM micrographs of purified CnoJOT origami are shown on the right. Scale bar, 100 nm.

## S3 Twisted revolution surfaces: model, parameters and helix routing algorithm, Figures S42 to S50

### S3.1 Initial helix spiraling route

Consider a continuously differentiable closed curve  $S : u \in [0, 1] \mapsto S(u)$  in the plane ( $\mathbf{XZ}$ ). Let us denote by  $\text{rot}_\theta^U$  denotes the rotation of angle  $\theta$  around the  $\mathbf{U}$ -axis. Consider a twist  $\tau \in \mathbb{N}$ . Let  $S_\tau^R(t, u) = \text{rot}_{2\pi t}^Z(R \cdot \mathbf{X} + \text{rot}_{\pi\tau t}^Y(S(u)))$  denotes the image of point  $S(u)$  rotated by  $\tau t$  half-turns around the  $\mathbf{Y}$ -axis, then translated by  $R$  along the  $\mathbf{X}$ -axis and finally rotated by  $t$  turn around the  $\mathbf{Z}$ -axis. The  $\tau$ -twisted revolution surface  $S_\tau^R$  of  $S$  with revolution radius  $R$  is defined as the set of points, see fig. S42:

$$S_\tau^R = \{S_\tau^R(t, u) : t, u \in [0, 1]\}$$

When  $\tau = 0$ ,  $S_0^R$  is simply the (untwisted) revolution surface with revolution radius  $R$ . When  $\tau$  is even or when  $\tau$  is odd and  $S$  has a half-turn symmetry,  $S_\tau^R$  is closed and continuously differentiable. The section  $S_\tau^R(t)$  of the twisted revolution surface at  $t$  turn (or, equivalently, at angle  $2\pi t$ ) refers to the set:

$$S_\tau^R(t) = \{S_\tau^R(t, u) : u \in [0, 1]\}$$

The designs considered in this paper correspond to:

- For the tori T1, . . . T6:  $S(u) = (2 \cos(2\pi u), 0, \sin(2\pi u))$  is the  $(2, 1)$ -ellipse and  $\tau$  ranges from 1 to 6;
- For the double-sphere DS:  $S$  corresponds to the  $\mathbb{C}$  curve and  $\tau = 0$ ;
- For the vault:  $S$  corresponds to the profile of the vault protein and  $\tau = 0$ .

Let  $P$  denotes the perimeter of  $S$ :

$$P = \int_u ||\dot{S}(u)|| du, \text{ where } \dot{S}(u) = dS(u)/du$$

We denote by  $s : \mathbb{R} \rightarrow \mathbb{R}$  the curvilinear abscissa of  $S$ :

$$s(x) = \int_{u=0}^x ||\dot{S}(u)|| du, \text{ such that } P = s(1)$$

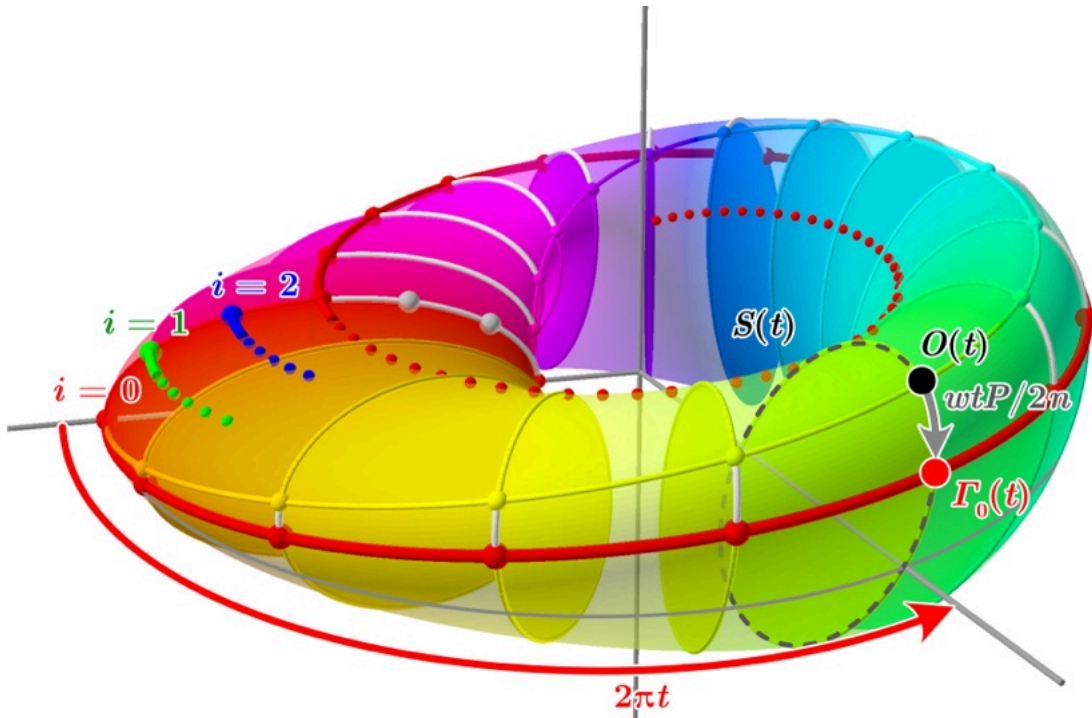


Figure S42: Initial helices routing.

and by  $\sigma = s^{-1}$  its reciprocal, such that  $\sigma(P) = 1$ .

**The initial spiral helix routing** consists in routing  $2n$  equally-spread helices per section according to a spiraling pattern. The number of helices is chosen to be even for allowing Given a revolution radius  $R$ , a section scale  $\gamma > 0$ , a winding parameter  $w \in \mathbb{Z}$ , the initial trajectories  $(\Gamma_i)$  passing through  $2n$  helices per section are defined:

$$\Gamma_i(t) = \gamma \cdot S_\tau^{(R/\gamma)}(t, \sigma((i + wt)P/2n)), \text{ for } t \in \mathbb{R} \text{ and } i = 0, 1, \dots$$

The principle is the following: on the initial section ( $t = 0$  turn) the  $2n$  helices are equally spread along the section, separated by  $P/2n$  measured along the section (before  $\gamma$ -rescaling). Then the distance measured along the section between them is kept constant but their position within the current section is shifted linearly as they rotate around the revolution axis, so that when a full turn is completed ( $t = 1$ ), they have moved by an integral number of  $P/2n$  (namely  $(i + w)$  times  $P/2n$ ), so that they connect to one of the initial helix, resulting into a set of spirals covering the whole target surface. The way the helices are connected together depends on the winding parameter  $w$ . Precisely: when  $\tau$  is even, Helix  $i$  connects to Helix  $(i + w) \bmod 2n$  after a full turn; whereas Helix  $i$  connects to Helix  $(i + n + w) \bmod 2n$  after a full turn when  $\tau$  is odd. It follows that Spiral  $i$  is the concatenation of all one-turn helix segment indexed in  $(i + w\mathbb{Z}) \bmod 2n$  when  $\tau$  is even, and by  $(i + (n + w)\mathbb{Z}) \bmod 2n$  when  $\tau$  is odd. If  $k = \gcd(2n, w)$  when  $\tau$  is even, and  $k = \gcd(2n, n + w)$  when  $\tau$  is odd,<sup>1</sup> then there are exactly  $k$  spirals making each exactly  $2n/k$  turns around the revolution axis. Note that if we want  $k$  to be even, for instance to ensure the existence of a proper scaffold routing, then we need  $w$  to be even if  $\tau$  is even, and  $w$  to have the same parity as  $n$  if  $\tau$  is odd.

The winding parameter not only influences the number of spirals and their number of turns around the revolution axis, but also the curvatures of the helices and their fit to the surface. This influence will be explored in the next section. Finally, one could think that the section scale  $\gamma$  should be set to  $2nH/P$  so that the inter-spiral distance  $\gamma P/2n = H = 2.65 \text{ nm}$  equals the desired inter-DNA helix axis distance. However, as this inter-spiral distance is measured along the section, it does not reflect to the real distance between the neighboring spirals and it needs to be adjusted to the angle taken by the spirals against the sections, which in turns depends on  $\gamma$  and  $w$ . This is why we need a special rescaling algorithm detailed in the following section S3.2.

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<sup>1</sup>where  $\gcd(a, b)$  denotes the greatest common divisor of integers  $a$  and  $b$ .



## S3.2 Helix routing and scaling algorithm

As mentioned earlier, when the surface goes through abrupt changes of curvature, the helices hit the section with a tight angle and their distance along the section must be adjusted. This is for instance the case at the entrance of the tunnels in the double sphere designs or in the most twisted tori (T3 and above). As shown in fig. S44, the distance between consecutive helices measured along the sections must be increased when the helices hit the section with a tight angle. As a consequence, we need to scale up the sections accordingly. This in turn requires to adjust the revolution radius, until a proper set of parameter (section scale, revolution radius) is found so that the total helix length matches the target scaffold length. We have designed a special algorithm to solve this non-linear optimization system. Our algorithm relies on a coarse-grained finite element system mapped according to  $m$  equally spaced sections, typically  $m \sim 20-200$ , see fig. S43. Each of the  $k$  helices, each making  $q = 2n/k$  turns, is broken into  $qm$  segments joining as many masses sliding along the  $m$  sections. Within each section, the masses are linked by springs whose empty lengths ( $\ell_{ij}^0$ ) are adaptively adjusted so that the segments corresponding to the helices are equally spaced at distance  $H$  (by default  $H = 2.65$  nm) from each other as explained next. In order to ensure that the helices stay as straight as possible, we add an angular spring around each mass so that the angle between its two adjacent helix segments ( $\beta$  in fig. S43) is pushed toward  $180^\circ$ .

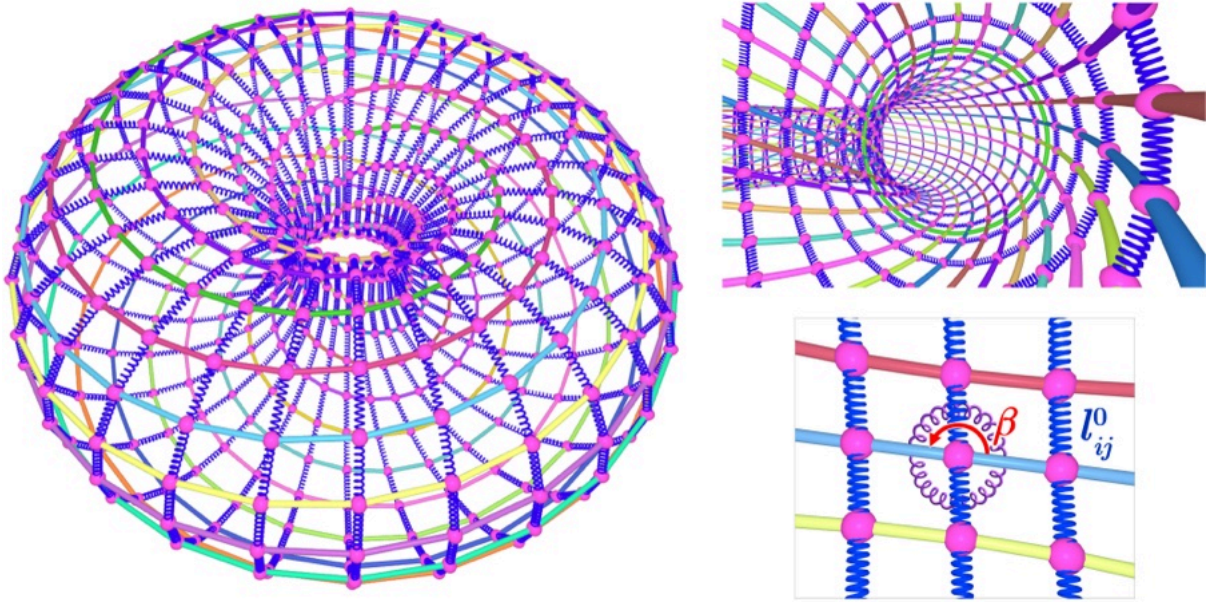


Figure S43: The coarse-grained finite element system used to optimize the helix trajectories.

**Our algorithm** proceeds as illustrated in fig. S44. It consists in two alternating phases: section scaling and revolution radius scaling, see fig. S44-C. During the section scaling, the revolution radius is fixed and the section are rescaled until the helices are  $H$  apart from each other. In order to do so, we let the masses converge to an equilibrium while adaptively adjusting the empty lengths of the springs to take into account the current incident angles of both segments of helices with the section: using the notations in fig. S44-C2, we want the distance  $d$  between two neighboring masses to verify that  $d \cos \alpha_1 \approx H \approx d \cos \alpha_2$ ; at every step of the simulation, we thus adjust the empty length of each spring to  $\ell_{ij}^0 := (H / \cos \alpha_1 + H / \cos \alpha_2) / 2$  and rescale the sections accordingly so that their perimeters are on average equal to the sum of the empty length of their springs. When the equilibrium is reached, we turn to the revolution radius phase: as illustrated in fig. S44-C2, the algorithm uses Chebyshev interpolation to compute a continuous trajectory for each of the  $k$  helices, compute their lengths in base pairs (rounding to the nearest integer) If the total length equals to the target scaffold length, the process ends. Otherwise the revolution radius is adjusted (reduced if the total length is higher, increased otherwise) and the algorithm goes back to the section rescaling phase.

As the process may not converge to the exact length of the scaffold, the user may interrupt the process at any time and the algorithm enforce the total length to the length of the scaffold by tuning a *minima*



1298 the rise of the  $k$  helices. This is useful without any harm when the process bounces between two integers  
 1299 surrounding the target value (e.g. between 7248 and 7250 when the target length is 7249).

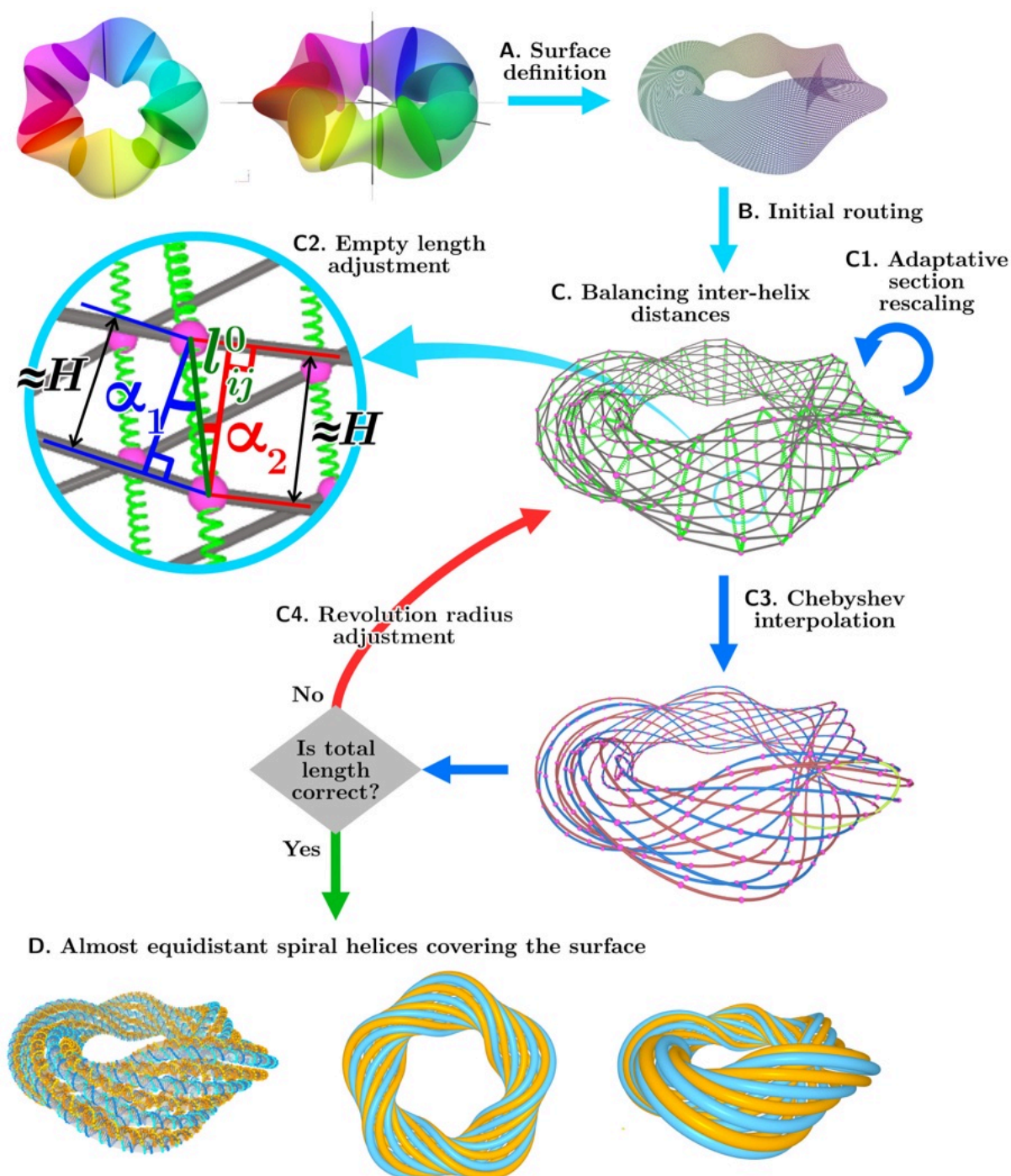


Figure S44: Helix routing and scaling algorithm

### S3.3 The winding parameter

The winding parameter controls the coiling and super-coiling of the spirals. Indeed, when Helix  $i$  completes a full turn, it completes  $\tau/2 + w/2n$  turns (super-coils) around the section axis. As shown in fig. S45, adjusting this parameter allows to select a set of initial trajectories that fits the surface while minimizes their torsion and curvature, and thus hopefully maximizing their foldability. ENSnano allows to tune this parameter to find the best winding parameter suited for your target surface.

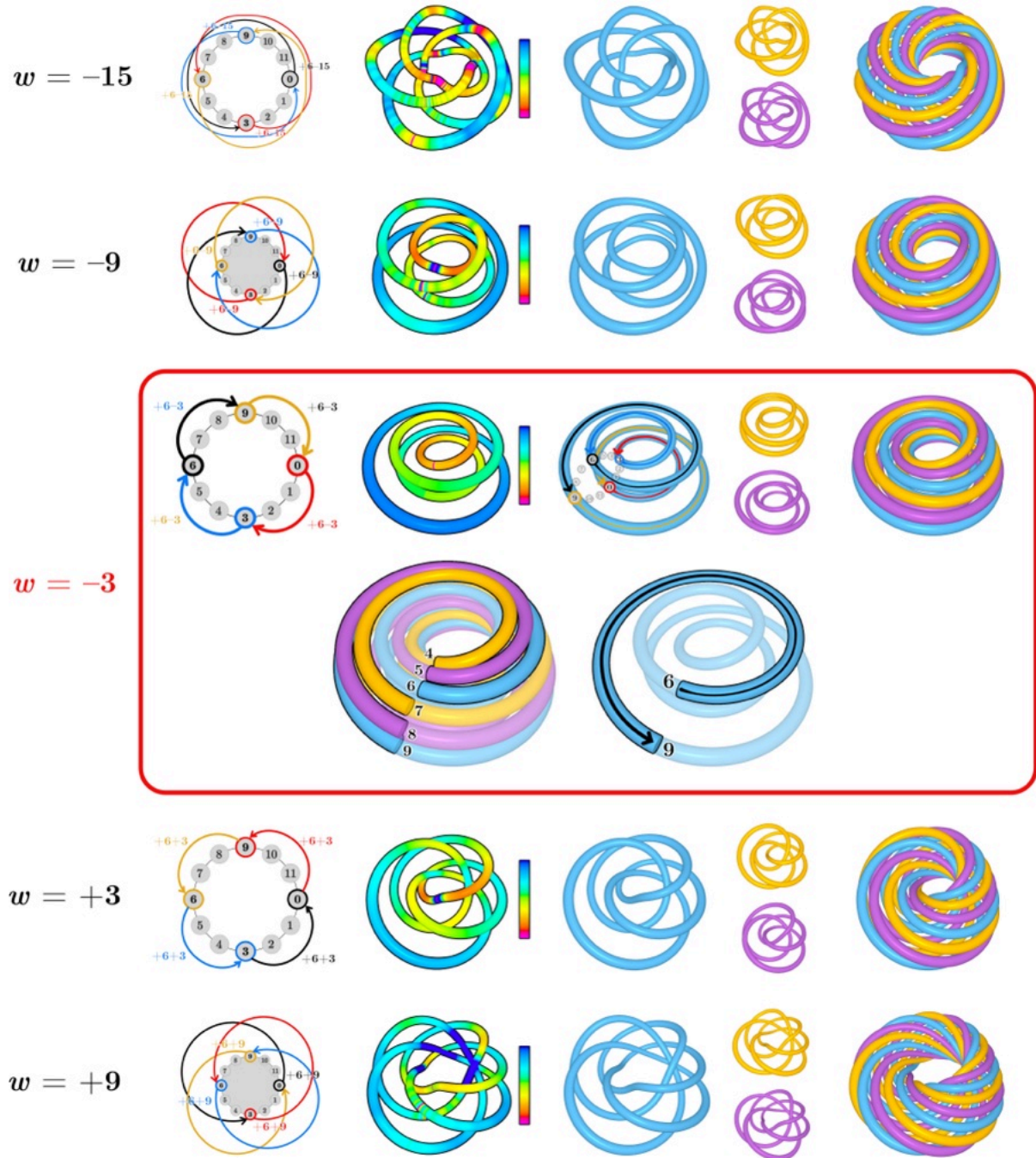


Figure S45: Influence of the winding parameter on the coiling, supercoiling and curvature of the helix spiraling trajectories in a virtual design with 12 = 2n helices per section. Setting  $w = +3$  produces the smoothest trajectories. Curvature color ranges from 5 nm to 20 nm.

### 1306 **S3.4 Design of twisted surfaces of revolution in ENSnano**

1307 (Un)twisted surfaces of revolution can be designed in ENSnano using the dedicated “Revolution Surface”  
1308 tab through the following workflow:

- 1309 1. Select the cross-sectional shape: an ellipse, a C-shape, or a closed Bézier path; and specify its geo-  
1310 metric parameters (radii, etc.).
- 1311 2. Specify the number of twists as a fraction of a full turn, according to the rotational symmetry of the  
1312 selected cross section.
- 1313 3. Set the revolution radius (positive or negative), either by entering its value numerically or by moving  
1314 the blue dashed rotation axis in the 3D view.
- 1315 4. Specify the number of helices intersecting each cross section.
- 1316 5. Define the helix routing by selecting the number of resulting spirals and the winding parameter.
- 1317 6. Specify the target total size, that is, the total number of base pairs in the structure.
- 1318 7. Click “Start” to launch the automatic helix-routing and shape-scaling procedure. The progress to-  
1319 wards equilibrium is displayed below the control buttons, including the current total scaffold length  
1320 and the variation in interhelix distances. The optimization can be interrupted at any time by press-  
1321 ing either “Abort”, which cancels the whole procedure, or “Finish”, which retains the current helix  
1322 routing and adjusts the DNA parameters to match the target scaffold size. The “Finish” option is  
1323 primarily intended for situations in which the system becomes trapped and oscillates between several  
1324 configurations that differ from the target scaffold size by only a few base pairs.

1325 Figure S46 provides a detailed overview of the ENSnano graphical interface for designing (un)twisted  
1326 surfaces of revolution.



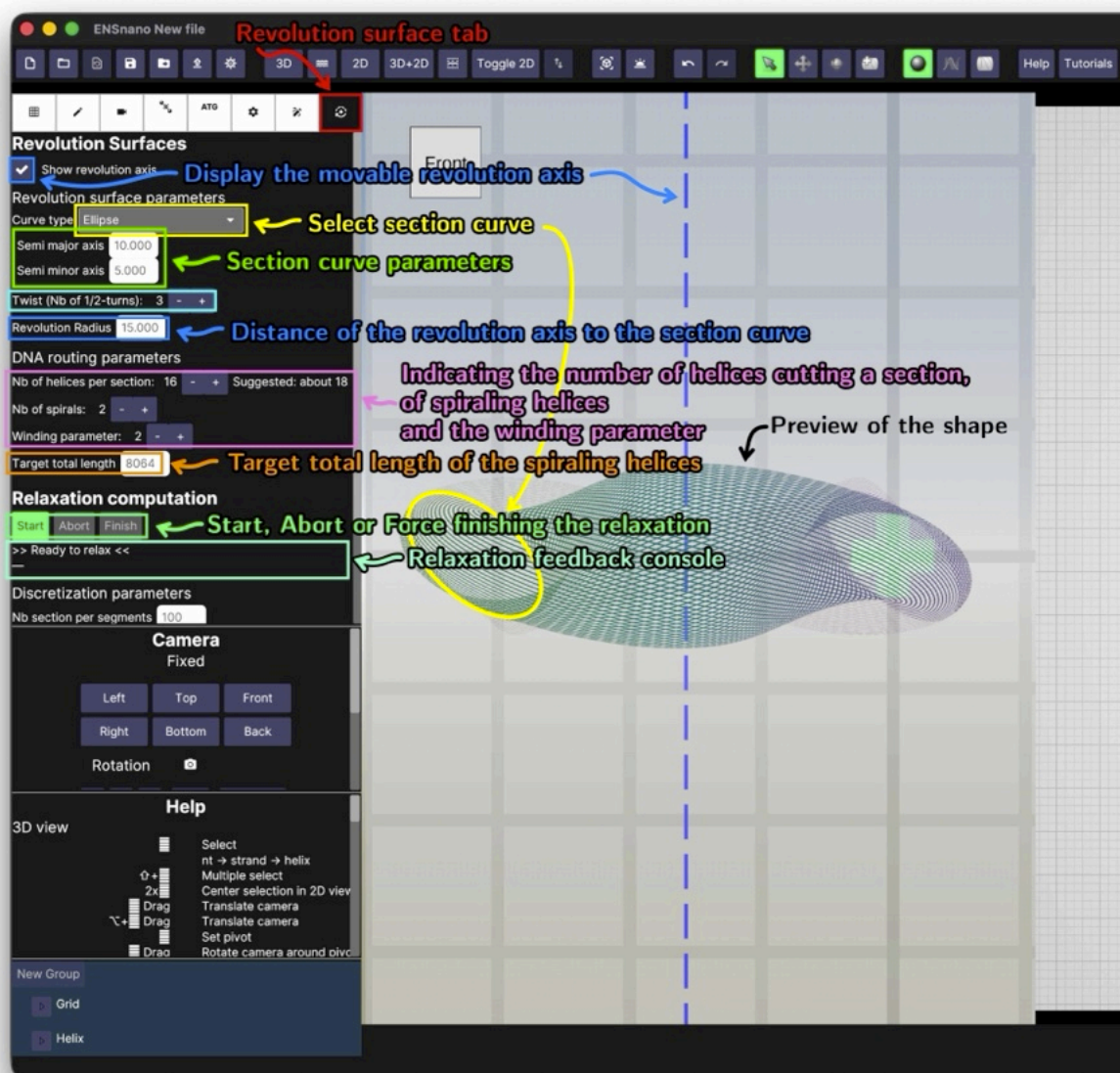


Figure S46: ENSnano surface-of-revolution design interface. From top to bottom: (1) *Surface of Revolution* tab. (2) *Display of the interactive axis of revolution*. (3) *Surface definition parameters*: selection of the cross-sectional shape and its geometric parameters, number of twists, and radius of revolution. (4) *DNA helix-routing parameters*: number of helices intersecting each cross section, number of resulting spirals, winding parameter, and target total size in base pairs. (5) *Control panel*: Start, Abort, and Finish buttons together with the progress console displaying the current total length and interhelix-distance variations.

As an example, fig. S47 illustrates the step-by-step definition of the surface of revolution used to generate T3. The parameters used to define the surface of revolution underlying each origami structure are provided together with the corresponding design in the following sections.

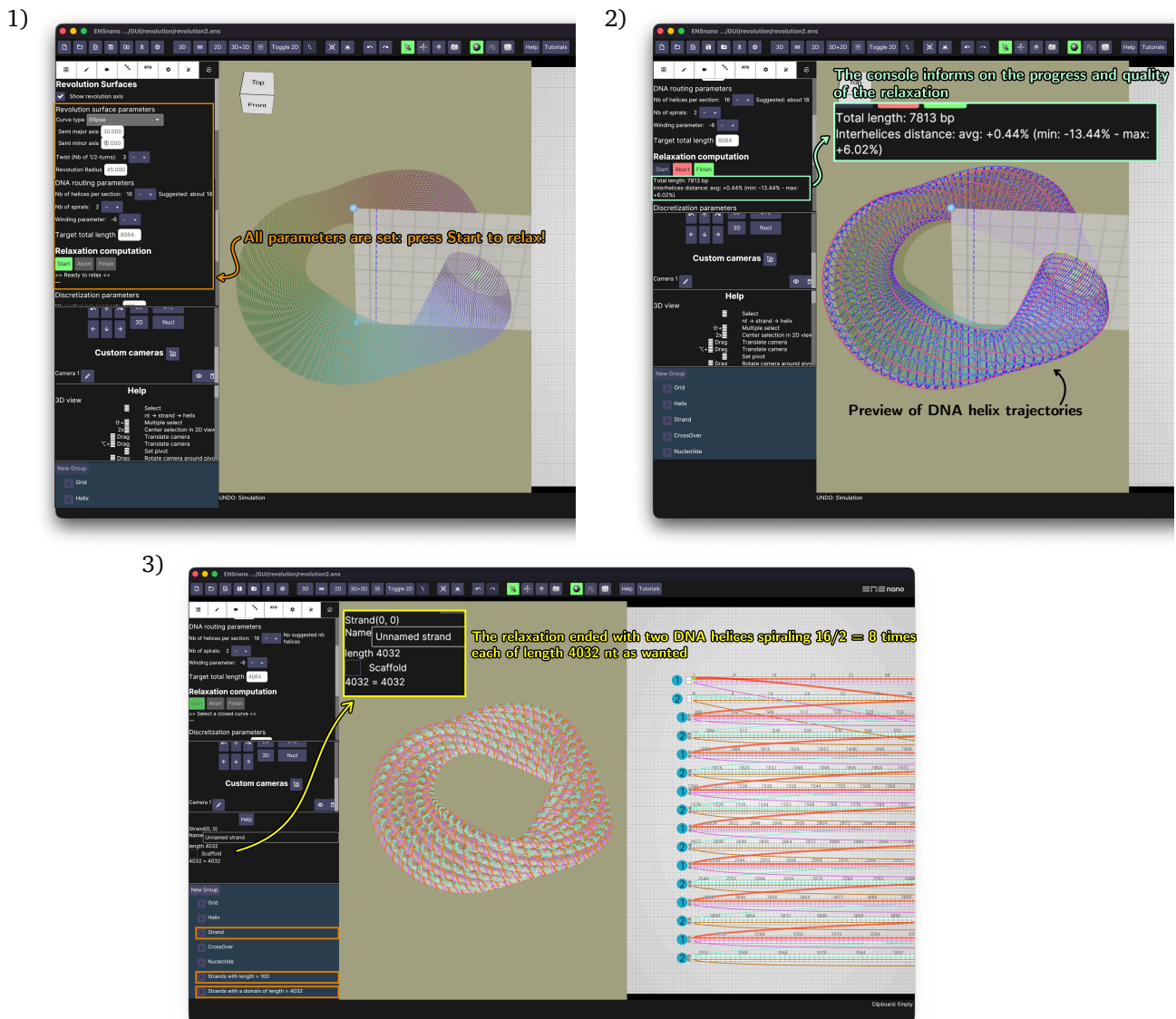


Figure S47: Step-by-step definition of a surface of revolution in ENSnano. (1) Define the geometric parameters of the surface: a  $30 \times 15$  elliptical cross section, a revolution radius of 45, and a  $3/2$  twist. Then specify the DNA helix-routing parameters: 16 helices per cross section, two spirals, a winding parameter of  $-6$ , and a target total size of 8064 base pairs. (2) Press “Start” and monitor the progress and quality of the helix-routing optimization in the console. (3) Resulting design. The optimized structure consists of two spiraling helices, each 4032 nucleotides long and completing eight turns around the revolution axis.



### S3.5 Torsion of the resulting helices as a function of the winding parameter

Figure S48 shows the average absolute value of the torsion measured along the resulting helix trajectories for the designs DS, and T1 to T6 for different values of the winding parameters (the closest possible around the chosen value for our designs). Observe that the winding parameter chosen for the origami design (marked with a red star) is the one minimizing the torsion along the helices. Observe as well that the torsion increases in designs T1 to T6 allowing to challenge our design paradigm with increasing curvature and torsion complexities.

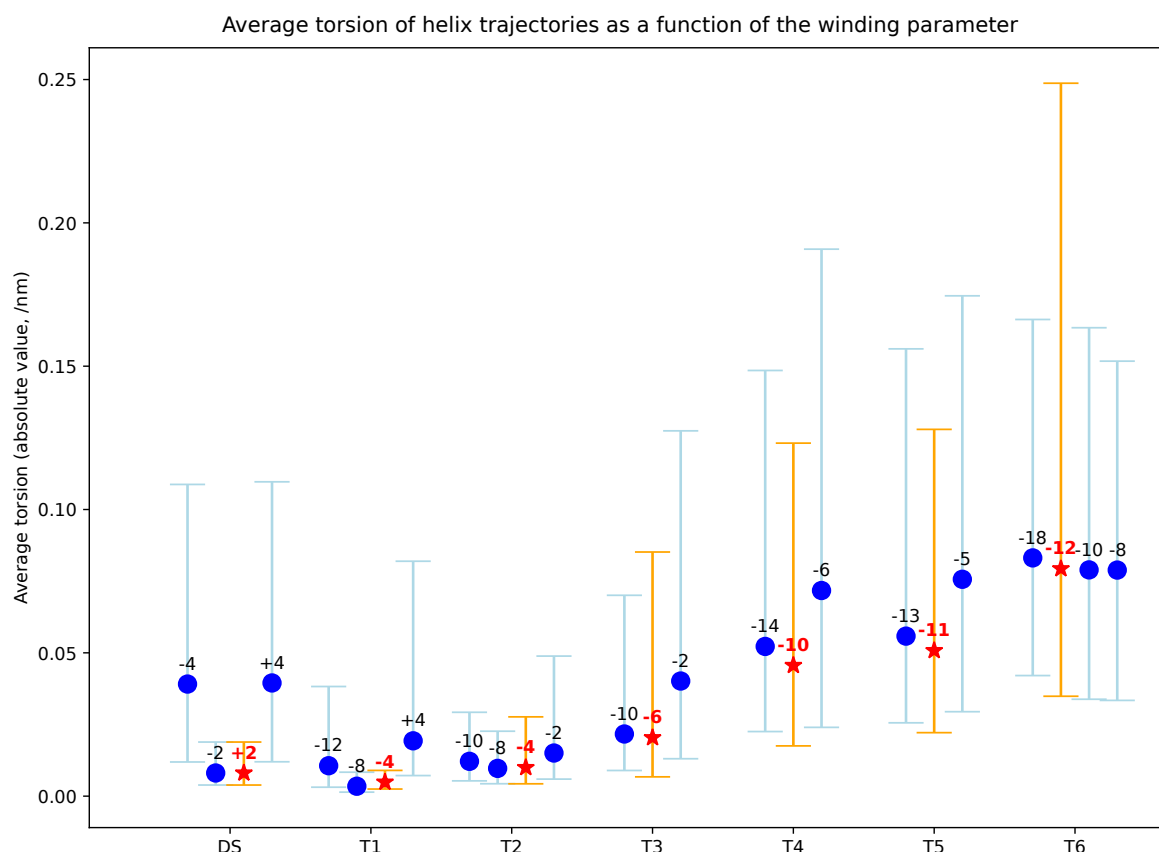


Figure S48: Average absolute torsion measured in  $\text{nm}^{-1}$  along the different helix trajectories as a function of the winding parameter  $w$  for the designs DS, and T1 to T6. The value of the winding parameter is indicated on top of each point. The chosen winding parameter is indicated by a red star for each design.

### S3.6 Impact on the helix trajectory torsion of the helix routing and scaling algorithm

Figure S49 shows the evolution of the average absolute torsion (in  $\text{nm}^{-1}$ ) of the helices trajectories computed during the execution of the helix routing and scaling algorithm (fig. S44). We observe that the angular springs (fig. S43) allow to reduce significantly the torsion of the helix trajectories during the first steps of the algorithm during which the inter-helix distances are balanced. The torsion then stabilizes while the algorithm finely tunes the shape scale to match the target scaffold length in the remaining steps.

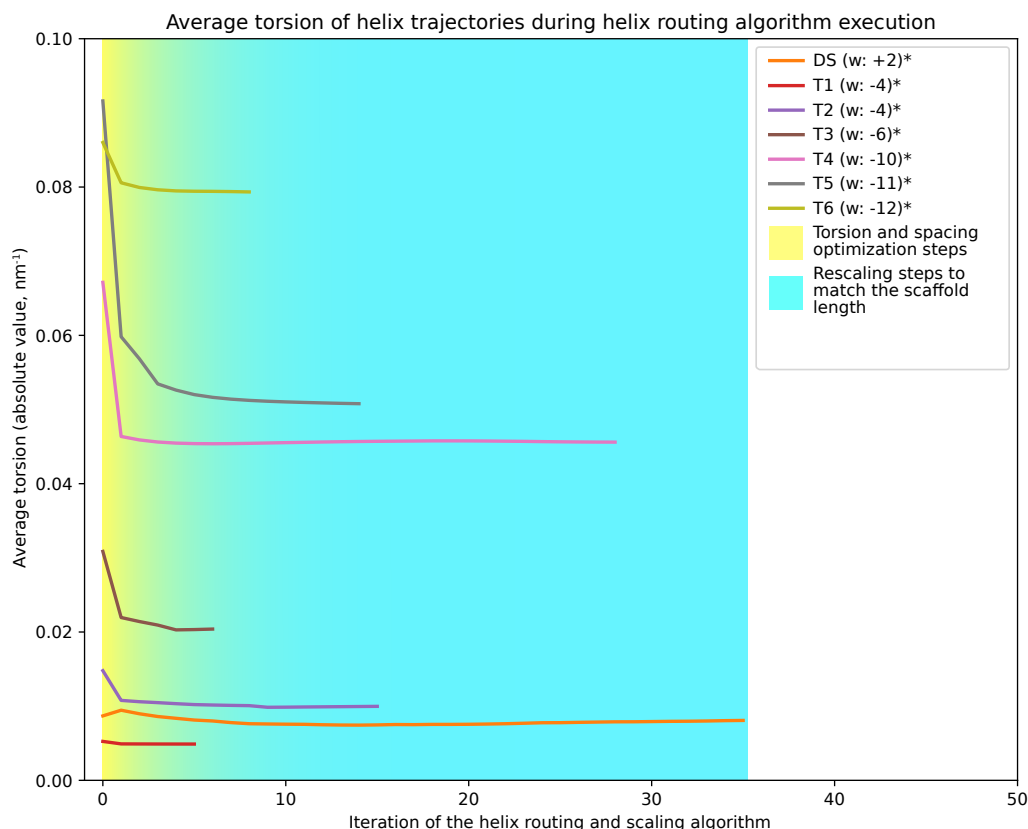


Figure S49: Evolution of the average torsion along the helices during the execution of the helix routing and scaling algorithm (fig. S44) for the designs DS and T1 to T6 with the chosen winding parameters. For every design, the torsion drops during the first stages of the algorithm during which the inter-helix distance are balanced and the trajectories are "rectified" and then the torsion remains quasi-constant during the second phase of the algorithm where the shape scale is finely tuned to reach the target scaffold length.

### S3.7 Torsion of the helix trajectories for all designs

Figure S50 shows the absolute value of the torsion measured along each helix trajectory for all the designs. Observe that the torsion in the vault and double-sphere DS reaches its peak in the tunnels where the helices sustain the highest slope change. Observe that the torsion increases significantly from T1 to T6, demonstrating that DNA helices sustain more and more strain from T1 to T6 (mostly in the inner circle).

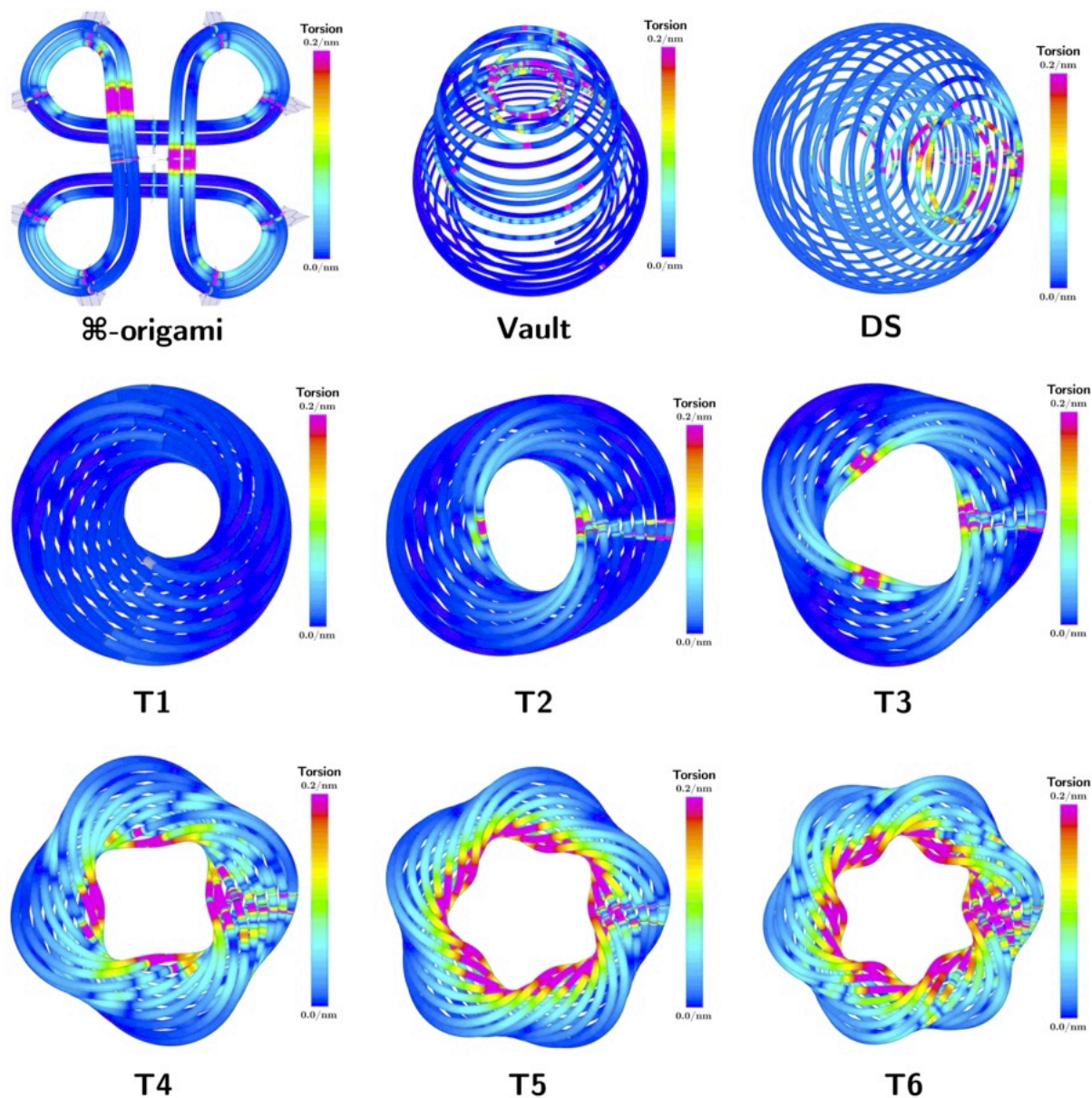


Figure S50: Absolute value of the torsion along each helix trajectory for all designs. Scale bar are uniform and range from  $0.0 \text{ nm}^{-1}$  (blue) to  $0.2 \text{ nm}^{-1}$  (purple).

## S4 Experimental validation of spiral-based design for hollow shaped toroidal surfaces, Figures S51 to S106

### S4.1 Tori designs

The tori described below, together with their underlying surfaces of revolution and helix-routing schemes, can be fully reproduced in ENSnano v0.7.3 or later using the parameters provided in the following sections.

#### S4.1.1 Torus T1 design

The twisted torus T1 is generated from a surface of revolution defined by a  $(1, 2)$  elliptical cross section and a  $1/2$  twist, with a helix routing comprising two helices that each make ten turns around the axis of revolution and a winding parameter of  $-4$ . The complete set of geometric and routing parameters is provided in fig. S51.

Revolution surface parameters

Curve type:

Semi major axis:

Semi minor axis:

Twist (Nb of 1/2-turns):  - +

Revolution Radius:

DNA routing parameters

Nb of helices per section:  - + Suggested: about 16

Nb of spirals:  - +

Winding parameter:  - +

Target total length:

Figure S51: Design parameters of torus T1 in ENSnano.



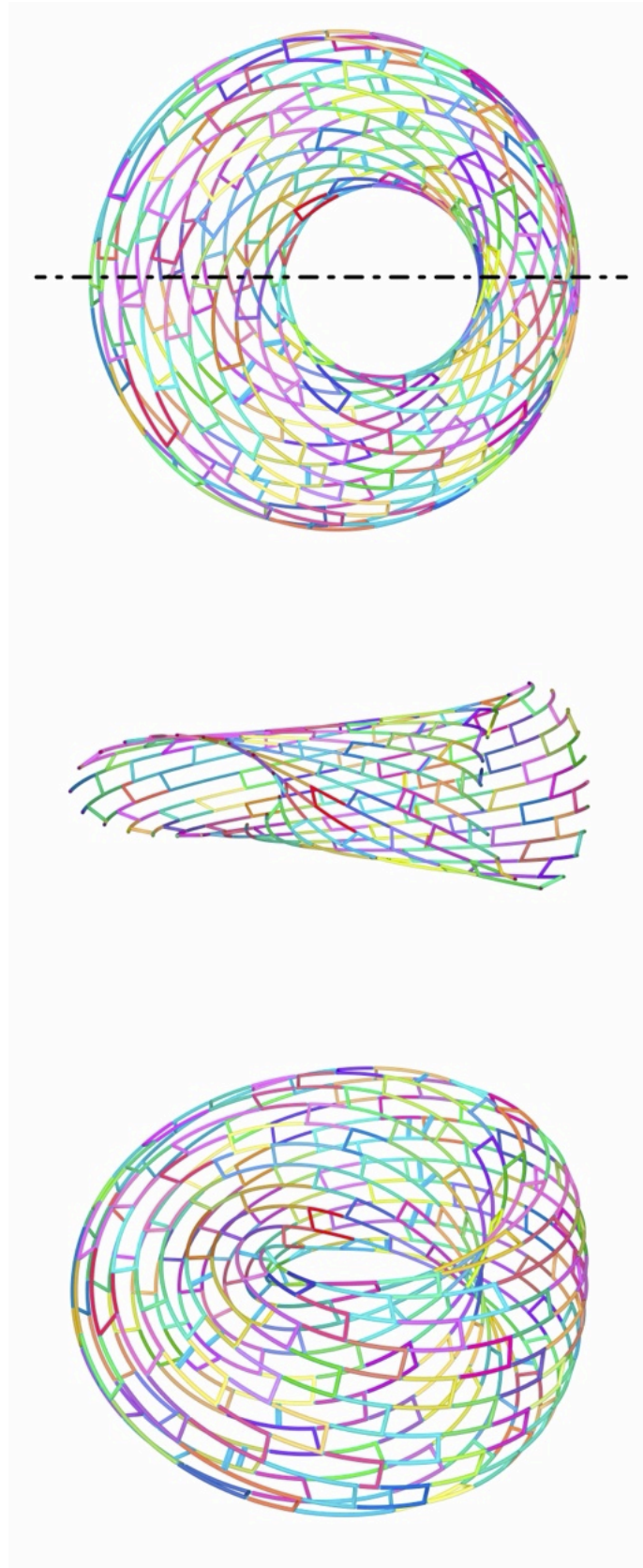


Figure S52: 3D view of the T1 design (line representation). From top to bottom: top view, cross-sectional view (along the dashed line indicated in the top view), and three-quarter view. For clarity, the scaffold strand is rendered with a slightly thinner line, leaving only its crossover pattern visible.

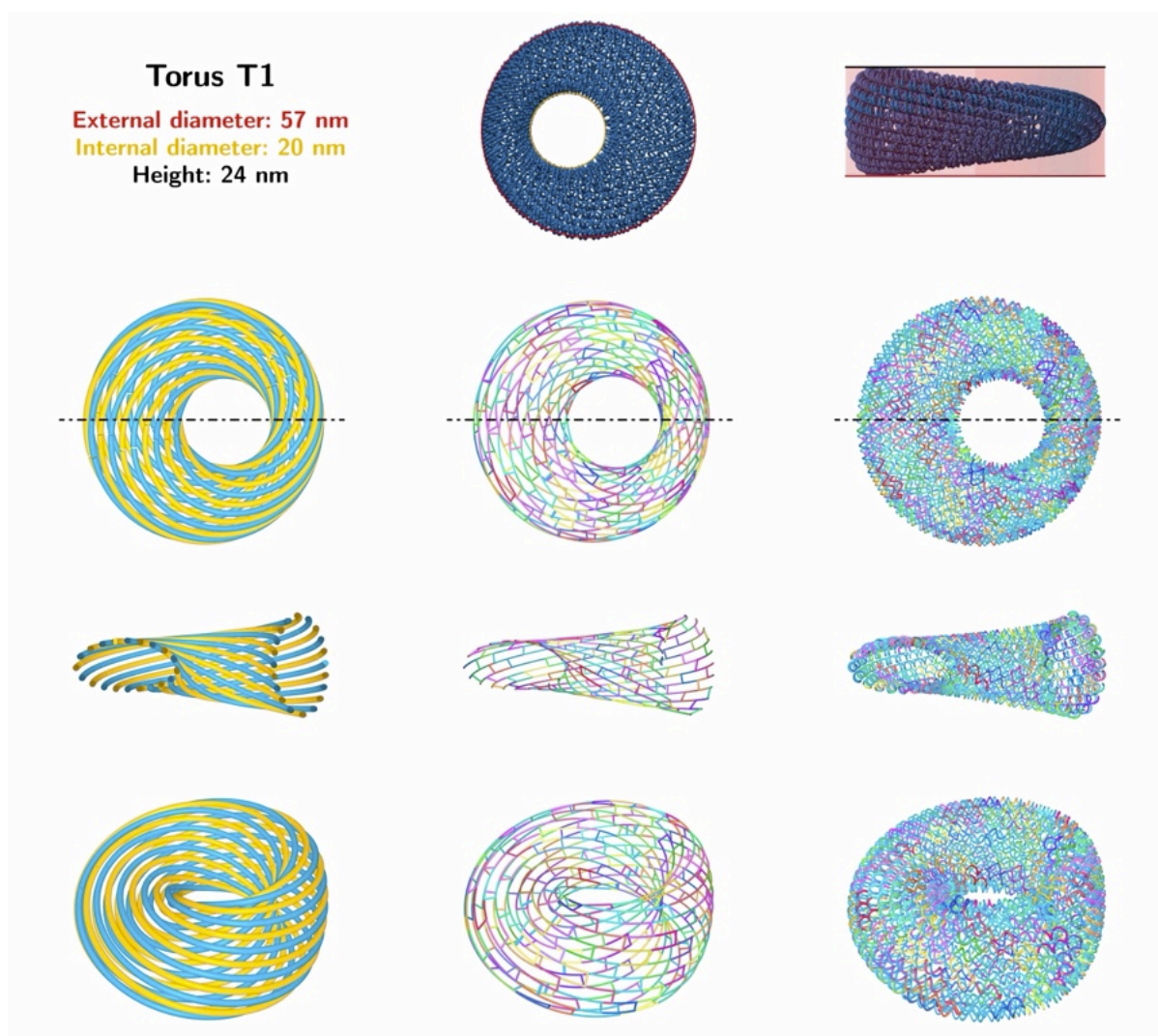


Figure S53: 3D views of the T1 design (helix, line, and full-strand representations). (Top row) Dimensions of the T1 design. The external and internal diameters are 57 nm (red) and 20 nm (yellow), respectively, and the height is 24 nm. (Other rows) From top to bottom: top view, cross-sectional view (along the dashed line indicated in the top view), and three-quarter view. From left to right: helix, line, and full-strand representations.

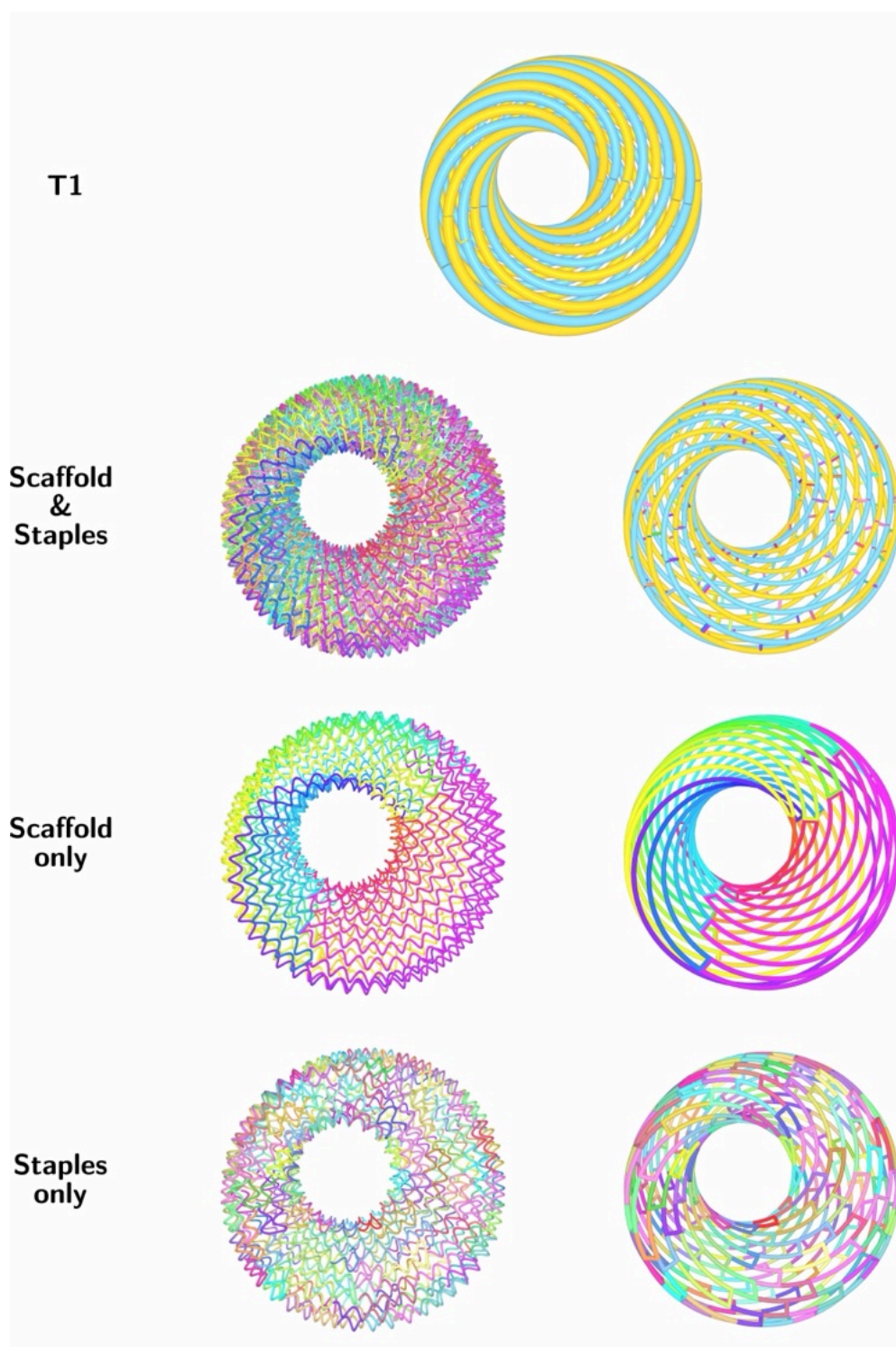


Figure S54: 3D views of the T1 design: helices, scaffold, and staples. (Top row) Helix representation. (Other rows) From top to bottom: all strands, scaffold strand only, and staple strands only. Left and right columns show the full-strand and line representations, respectively.



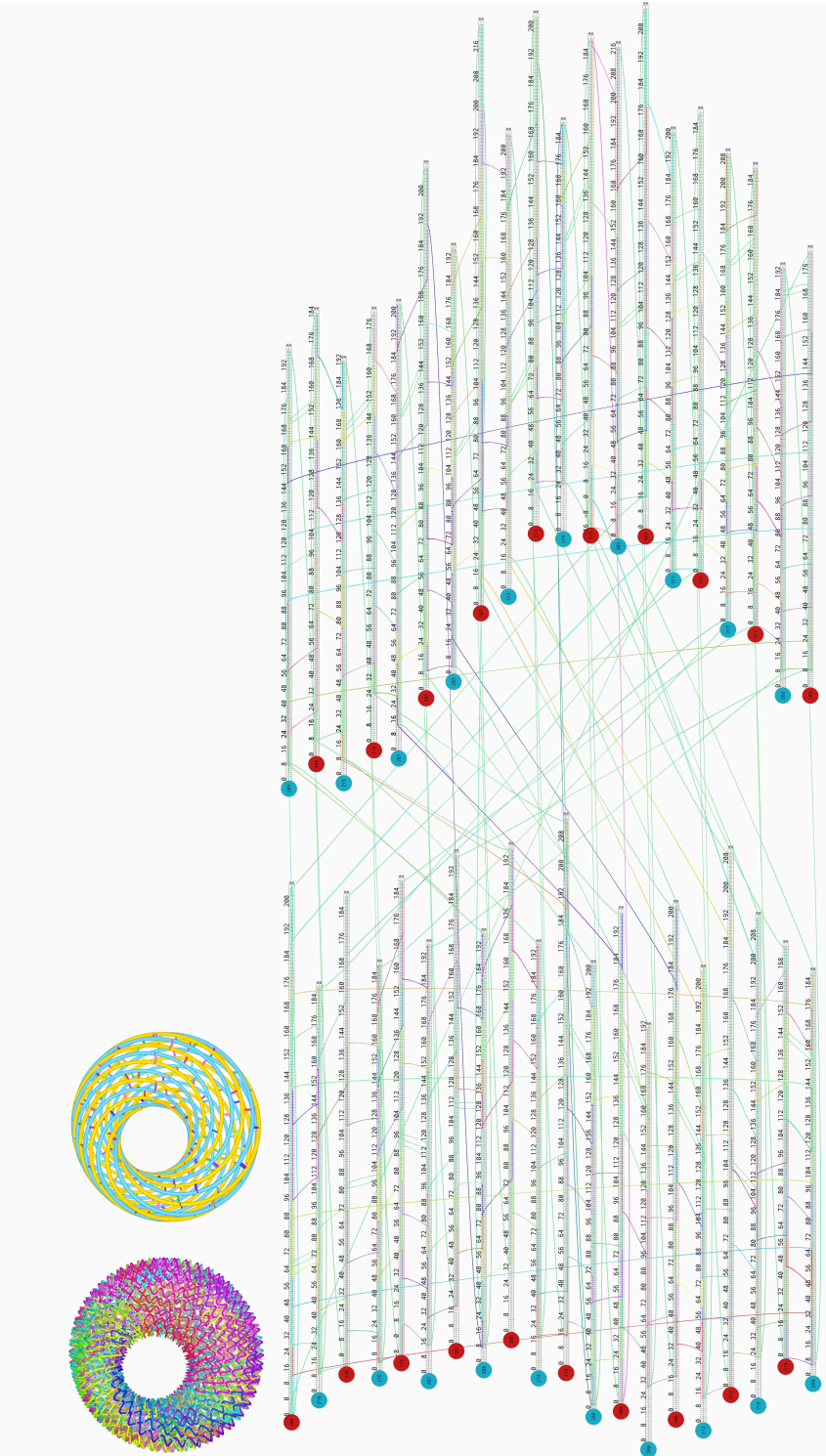


Figure S55: 2D view of the T1 design. Scaffold and staples.

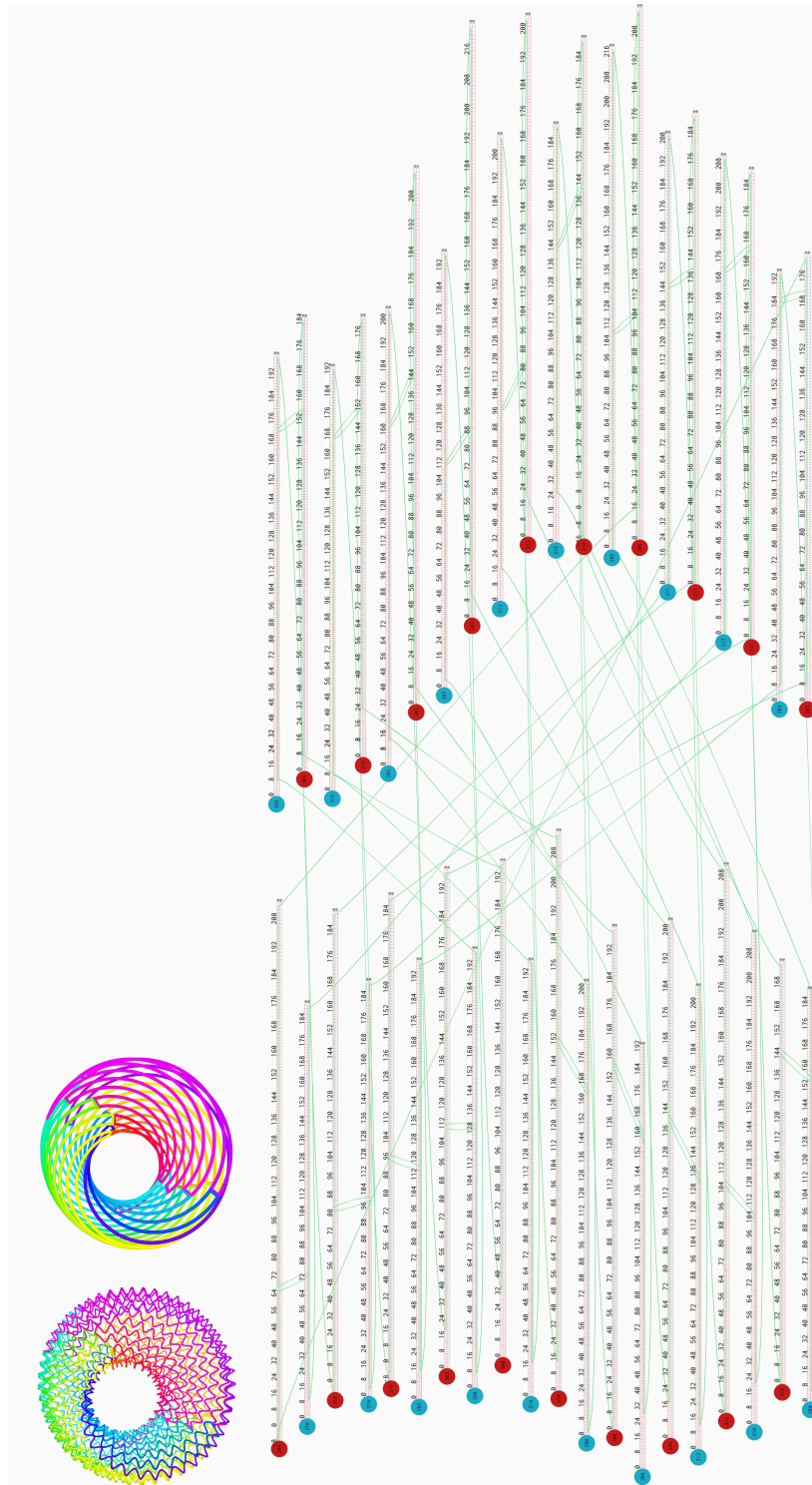


Figure S56: 2D view of the T1 design. Scaffold only.



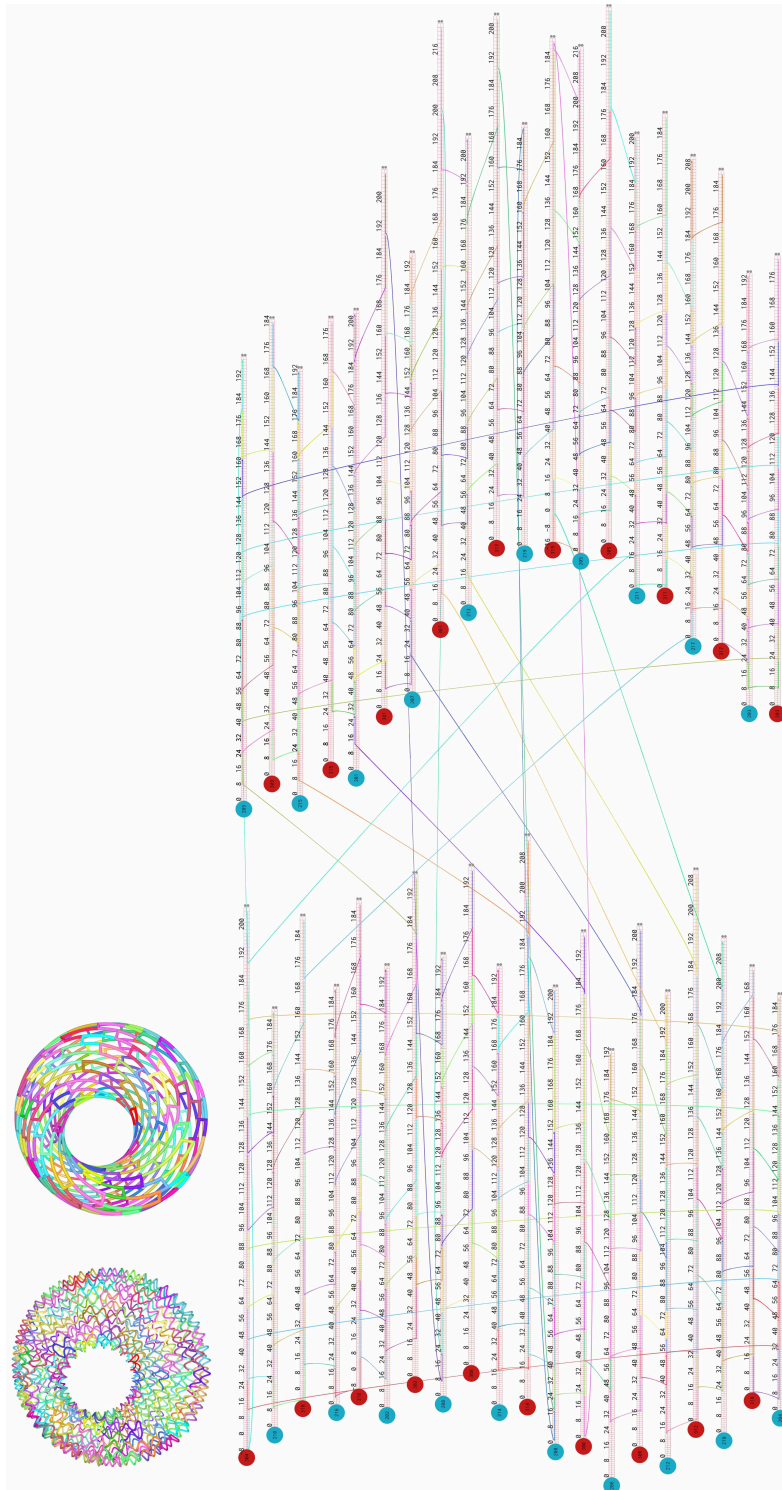


Figure S57: 2D view of the T1 design. Staples only.

### S4.1.2 Torus T2 design

The twisted torus T2 is generated from a surface of revolution defined by a  $(1, 2)$  elliptical cross section and a  $2/2$  twist, with a helix routing comprising two helices that each make nine turns around the axis of revolution and a winding parameter of  $-4$ . The complete set of geometric and routing parameters is provided in fig. S58.

Revolution surface parameters

Curve type: Ellipse

Semi major axis: 10

Semi minor axis: 5

Twist (Nb of 1/2-turns): 2 - +

Revolution Radius: 21.100

DNA routing parameters

Nb of helices per section: 18 - + Suggested: about 16

Nb of spirals: 2 - +

Winding parameter: -4 - +

Target total length: 7560

Figure S58: Design parameters of torus T2 in ENSnano.

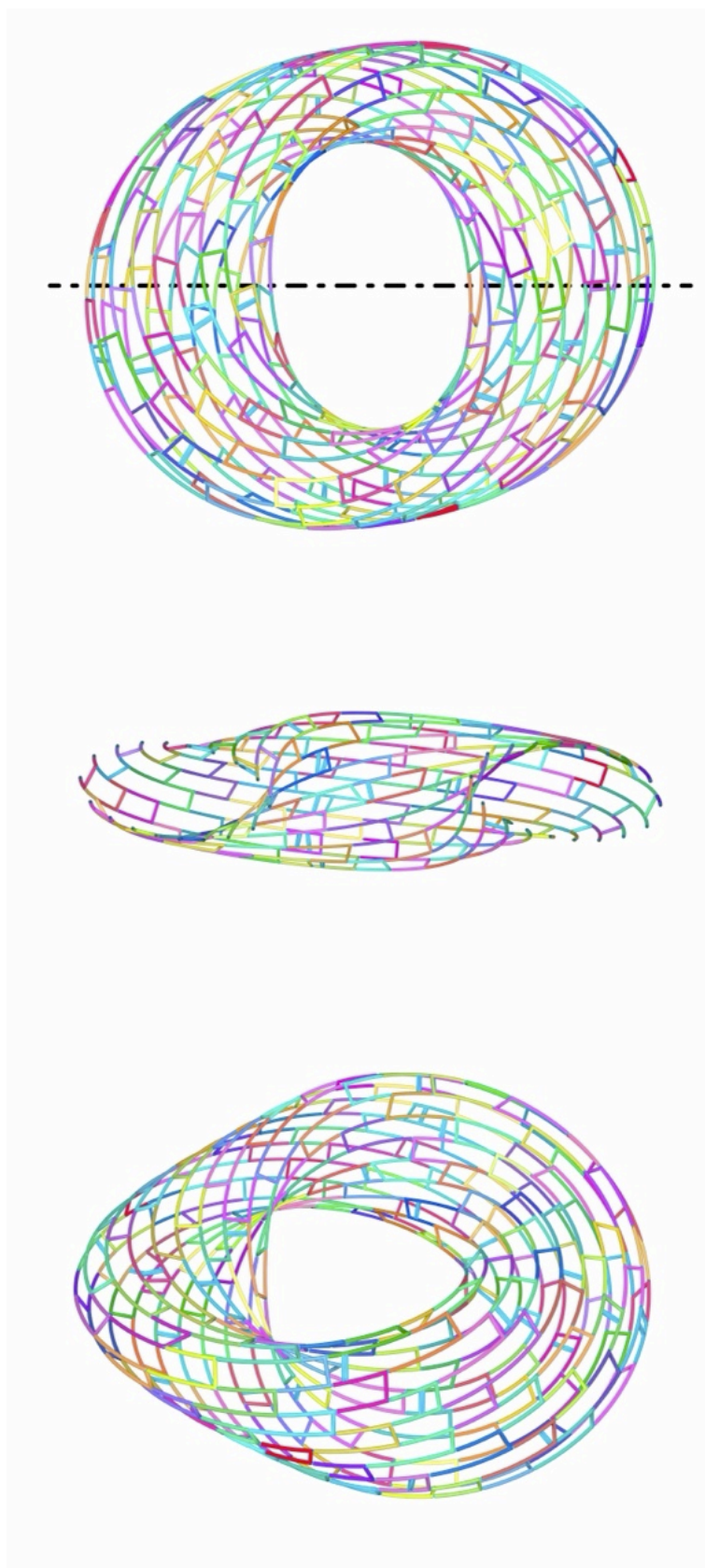


Figure S59: 3D view of the T2 design (line representation). From top to bottom: top view, cross-sectional view (along the dashed line indicated in the top view), and three-quarter view. For clarity, the scaffold strand is rendered with a slightly thinner line, leaving only its crossover pattern visible.

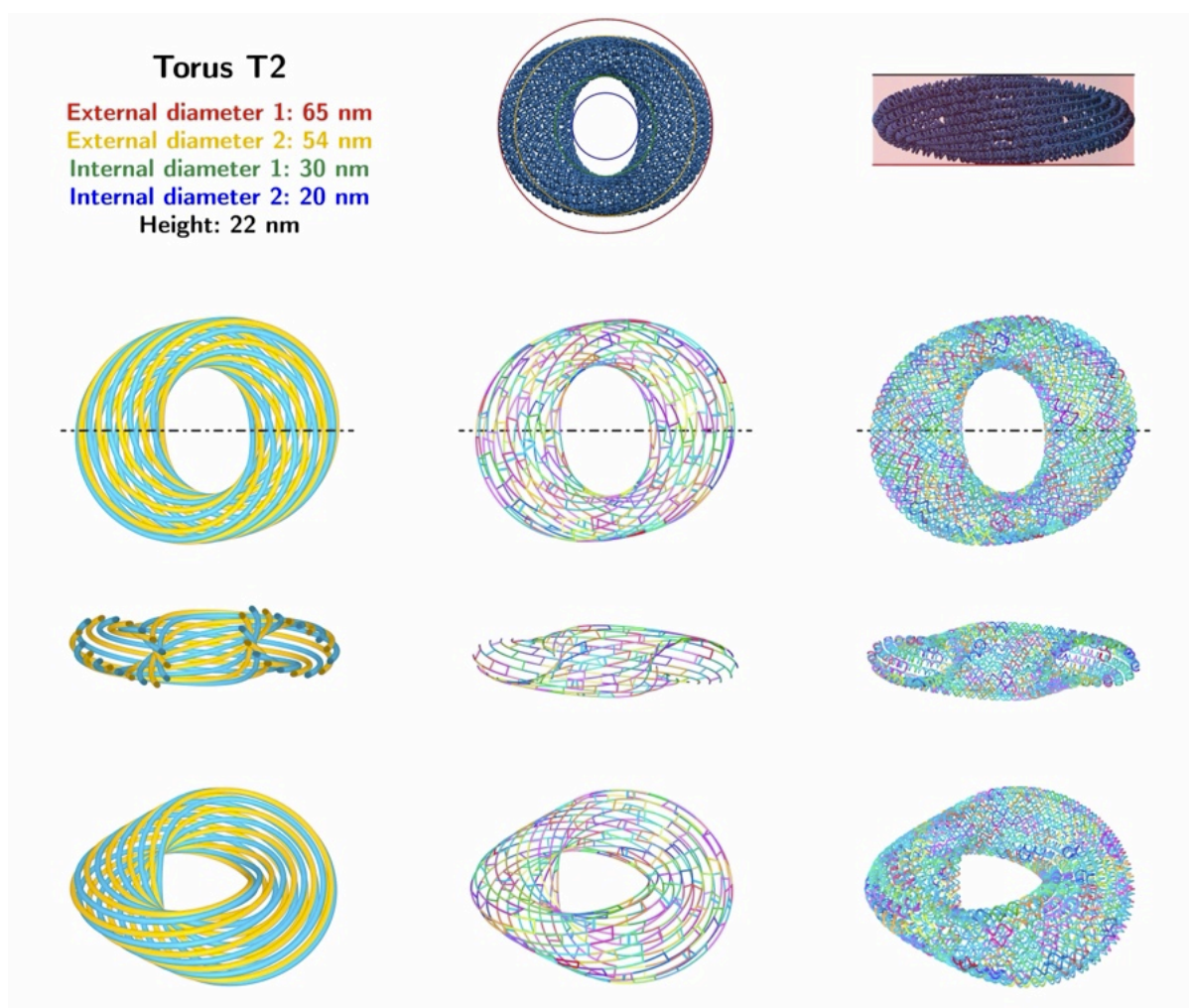


Figure S60: 3D views of the T2 design (helix, line, and full-strand representations). (Top row) Dimensions of the T2 design. The two external and two internal diameters are 65 nm (red), 54 nm (yellow), 30 nm (green) and 20 nm (blue), respectively, and the height is 22 nm. (Other rows) From top to bottom: top view, cross-sectional view (along the dashed line indicated in the top view), and three-quarter view. From left to right: helix, line, and full-strand representations.



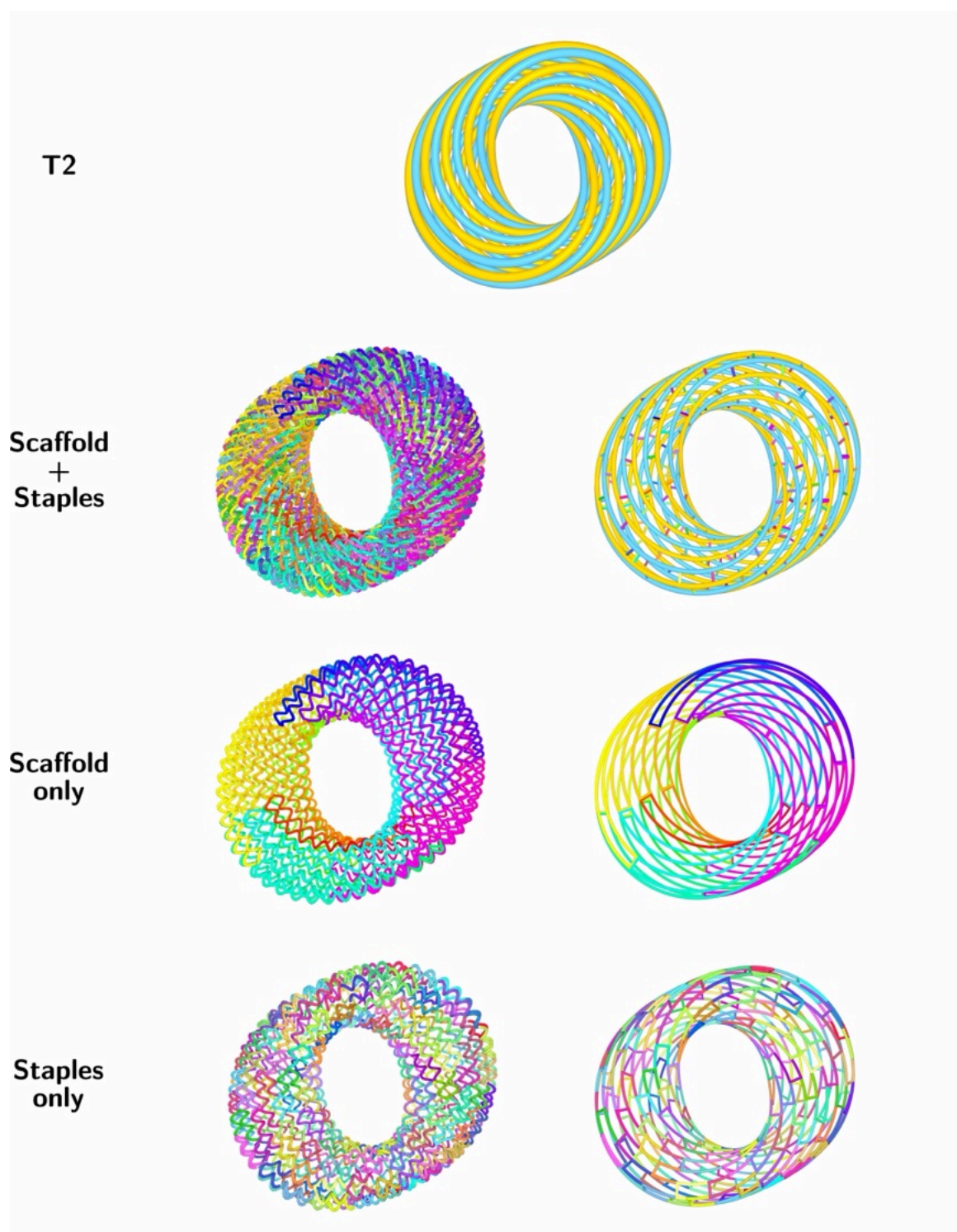
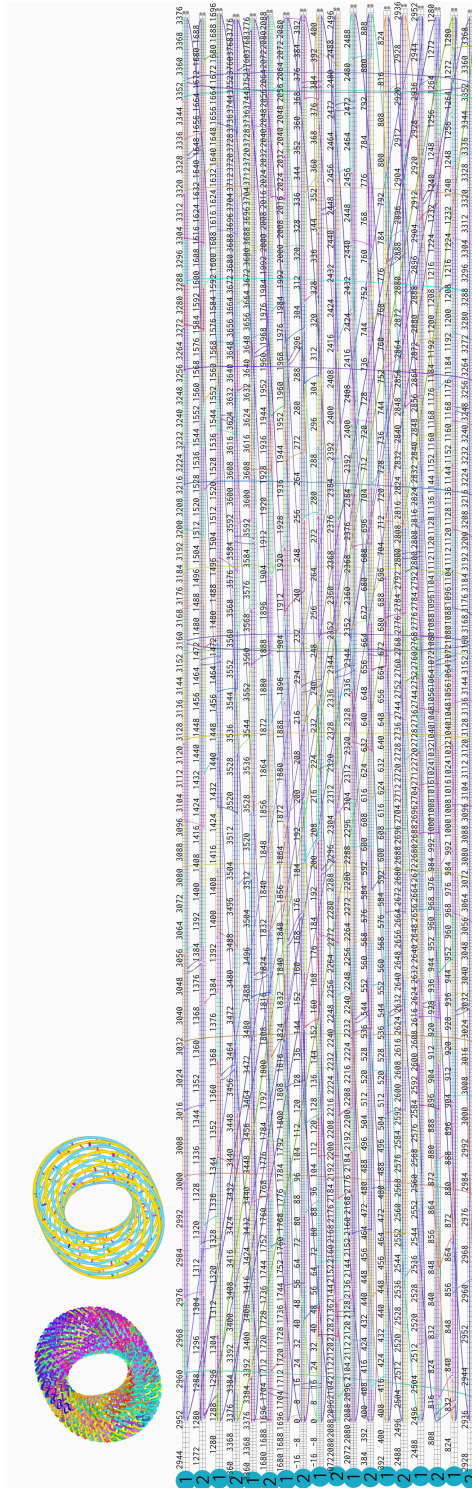
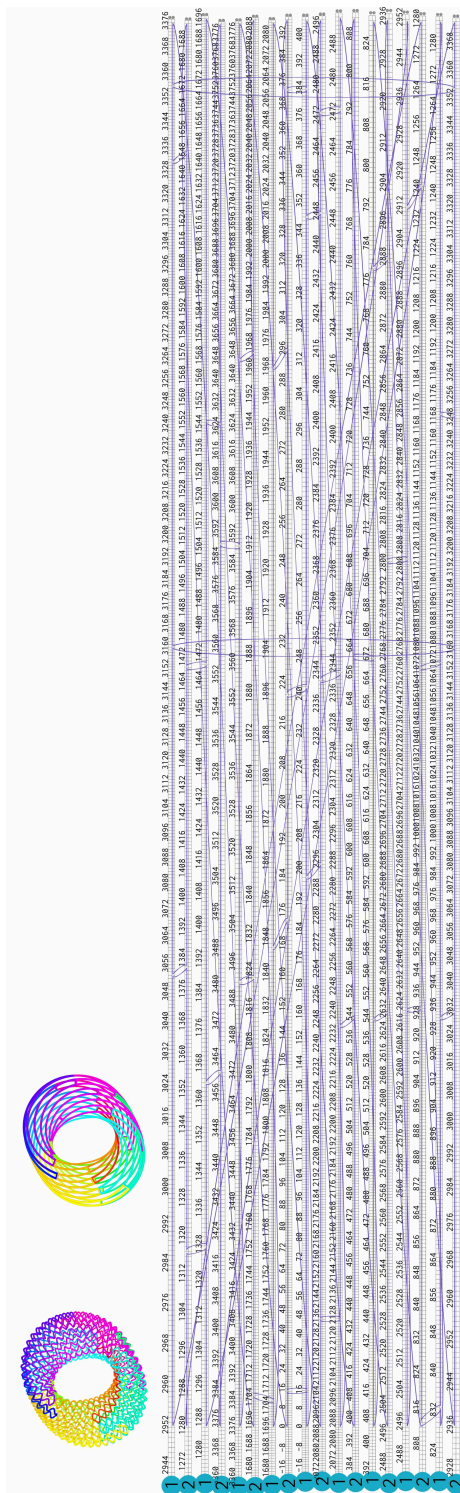


Figure S61: 3D views of the T2 design: helices, scaffold, and staples. (Top row) Helix representation. (Other rows) From top to bottom: all strands, scaffold strand only, and staple strands only. Left and right columns show the full-strand and line representations, respectively.







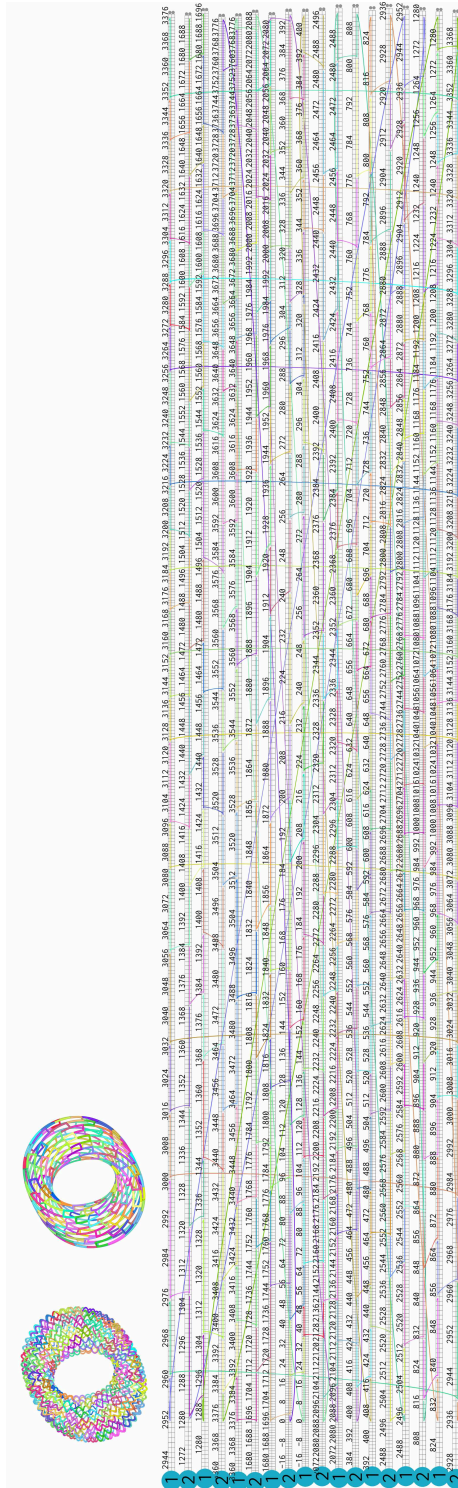


Figure S64: 2D view of the Torus T2 design. Staples only.



### S4.1.3 Torus T3 design

The twisted torus T3 is generated from a surface of revolution defined by a (1, 2) elliptical cross section and a 3/2 twist, with a helix routing comprising two helices that each make height turns around the axis of revolution and a winding parameter of  $-6$ . The complete set of geometric and routing parameters is provided in fig. S65.

Revolution surface parameters

Curve type:

Semi major axis:

Semi minor axis:

Twist (Nb of 1/2-turns):  - +

Revolution Radius:

DNA routing parameters

Nb of helices per section:  - + Suggested: about 14

Nb of spirals:  - +

Winding parameter:  - +

Target total length:

Figure S65: Design parameters of torus T3 in ENSnano.

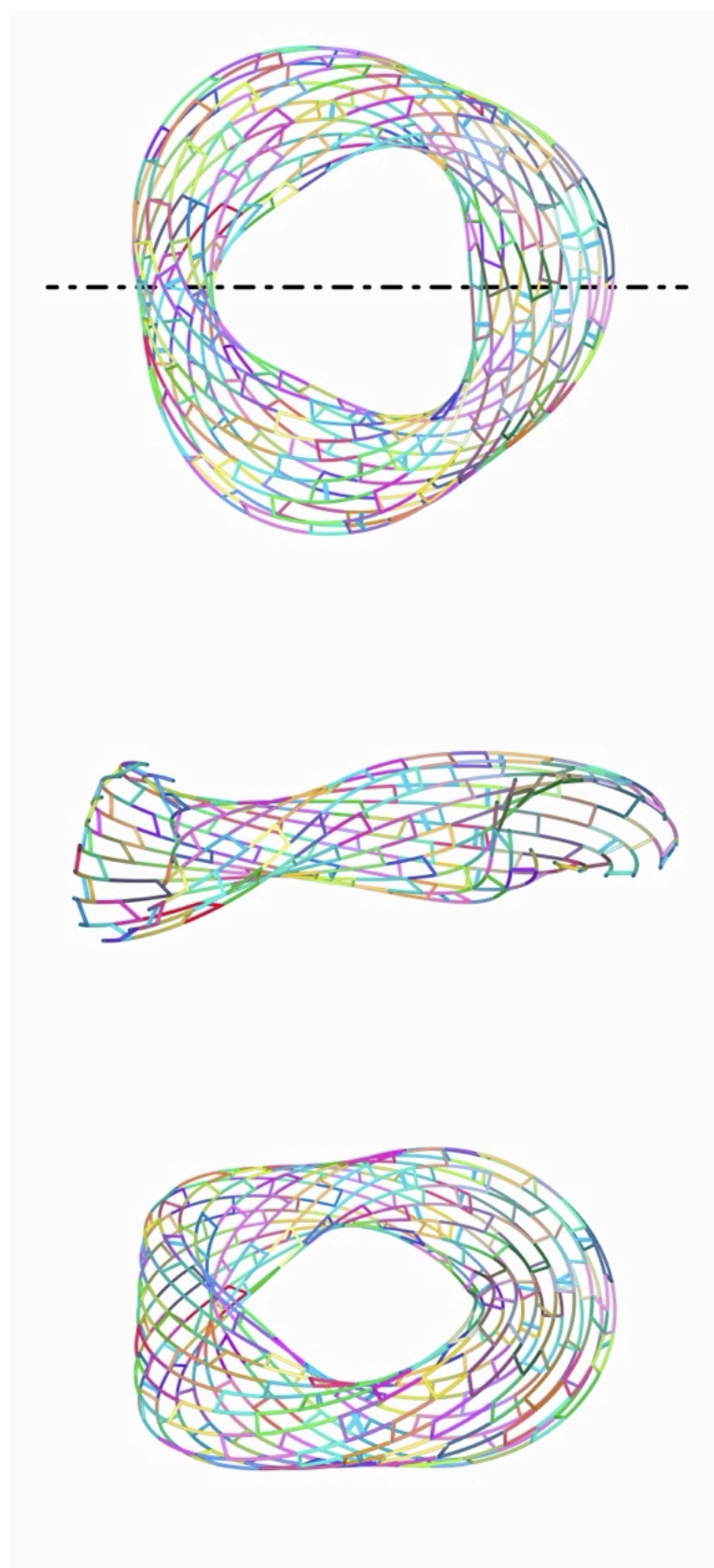


Figure S66: 3D view of the T3 design (line representation). From top to bottom: top view, cross-sectional view (along the dashed line indicated in the top view), and three-quarter view. For clarity, the scaffold strand is rendered with a slightly thinner line, leaving only its crossover pattern visible.

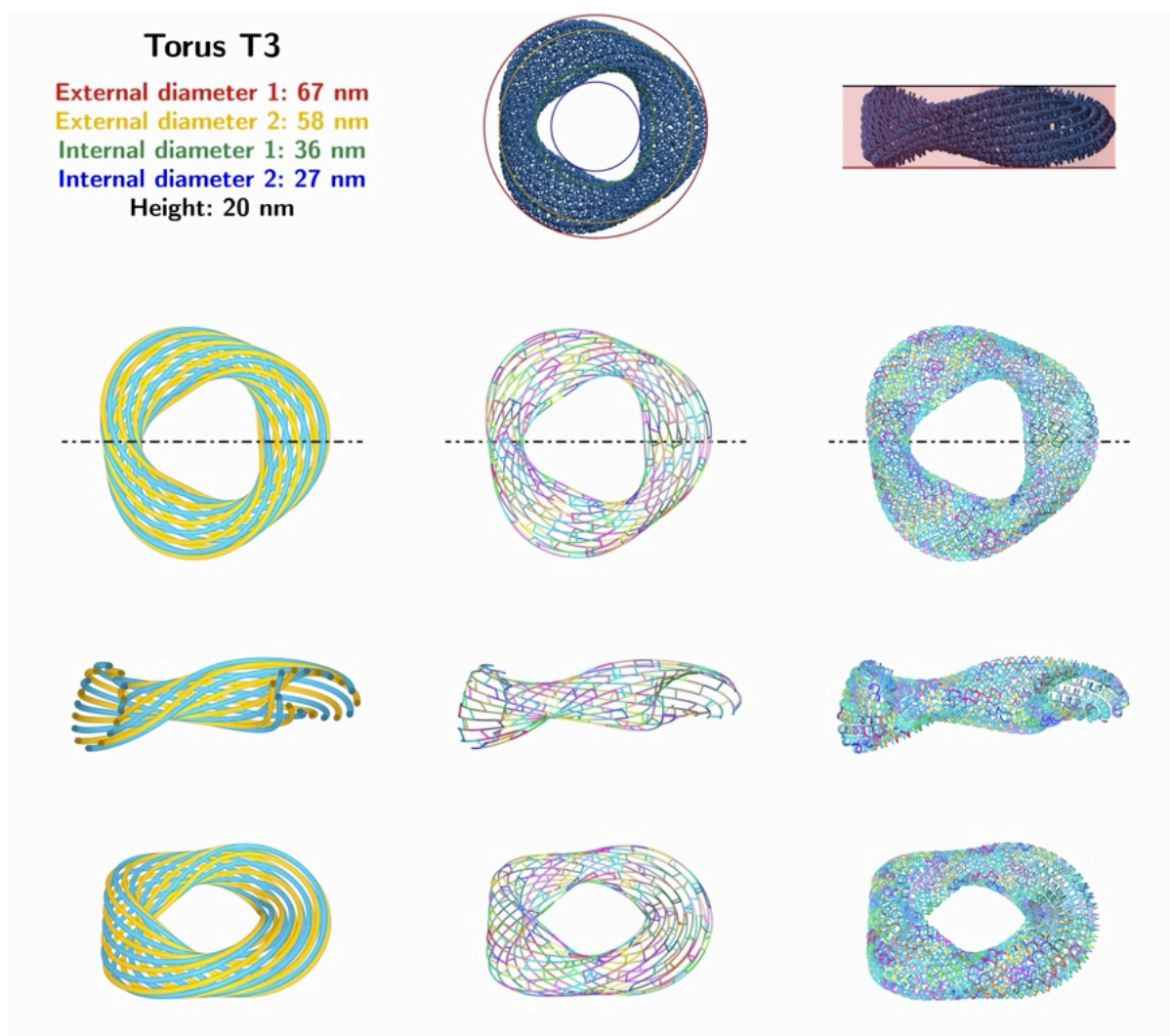


Figure S67: 3D views of the T3 design (helix, line, and full-strand representations). (Top row) Dimensions of the T3 design. The two external and two internal diameters are 67 nm (red), 58 nm (yellow), 36 nm (green) and 27 nm (blue), respectively, and the height is 20 nm. (Other rows) From top to bottom: top view, cross-sectional view (along the dashed line indicated in the top view), and three-quarter view. From left to right: helix, line, and full-strand representations.

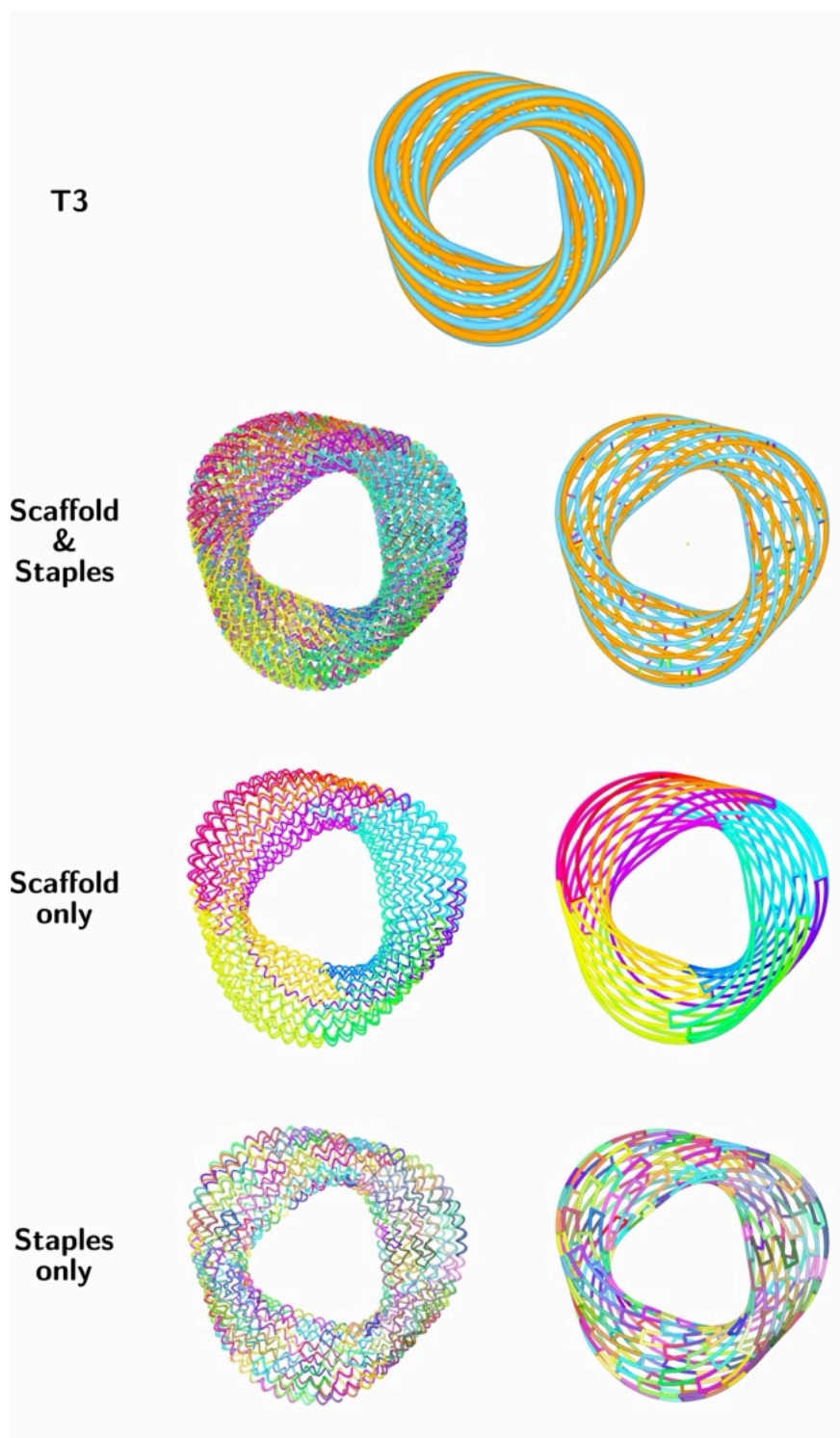


Figure S68: 3D views of the T3 design: helices, scaffold, and staples. (Top row) Helix representation. (Other rows) From top to bottom: all strands, scaffold strand only, and staple strands only. Left and right columns show the full-strand and line representations, respectively.



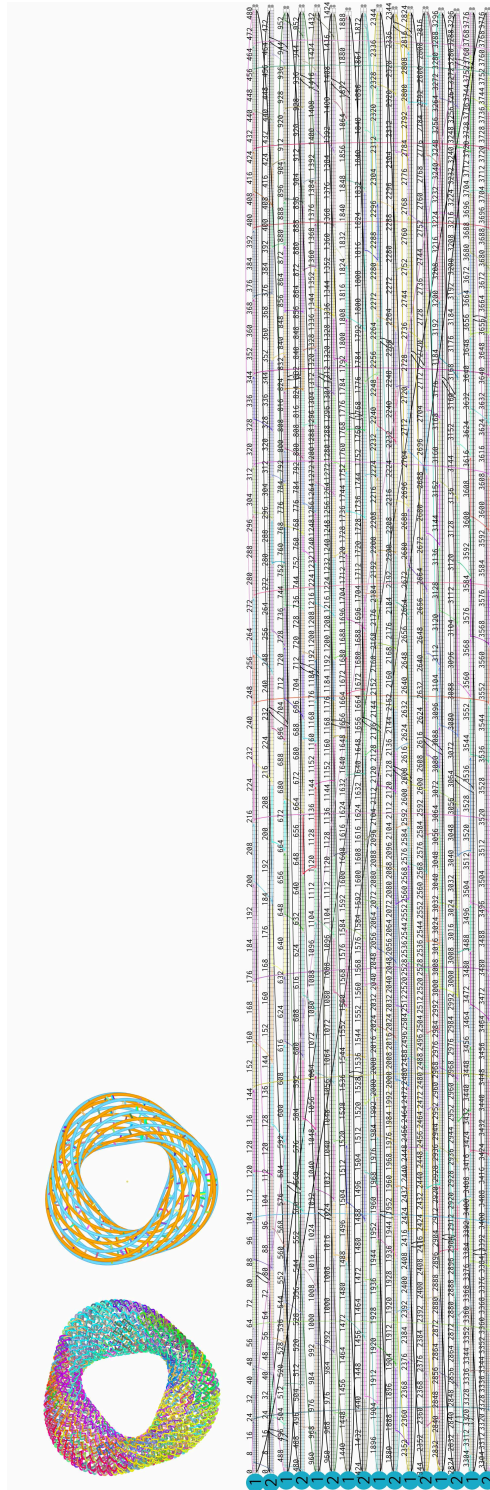


Figure S69: 2D view of the Torus T3 design. Scaffold and staples.

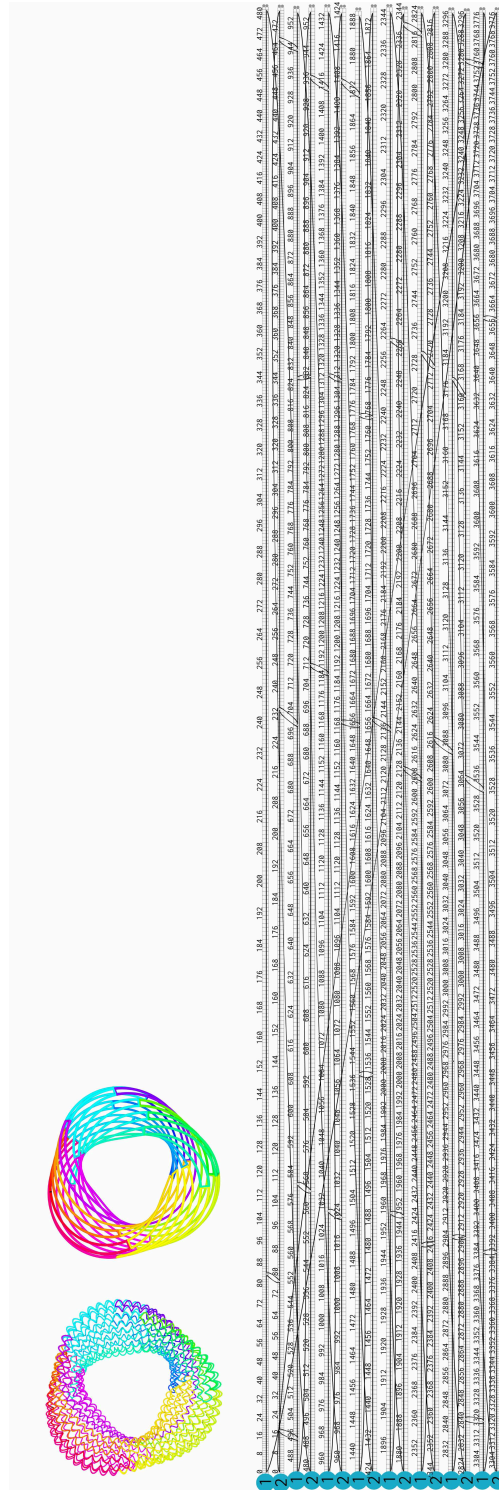


Figure S70: 2D view of the Torus T3 design. Scaffold only.

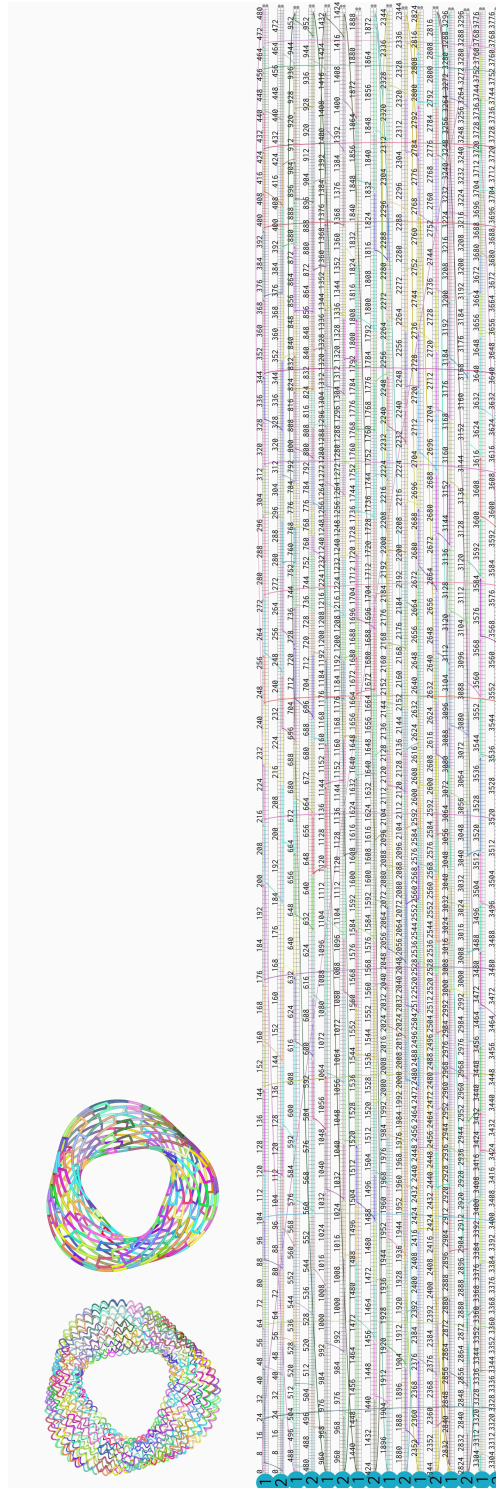


Figure S71: 2D view of the Torus T3 design. Staples only.

#### S4.1.4 Torus T4 design

The twisted torus T4 is generated from a surface of revolution defined by a  $(1, 2)$  elliptical cross section and a  $4/2$  twist, with a helix routing comprising two helices that each make nine turns around the axis of revolution and a winding parameter of  $-10$ . The complete set of geometric and routing parameters is provided in fig. S72.

Revolution surface parameters

Curve type: Ellipse

Semi major axis: 9.6

Semi minor axis: 4.8

Twist (Nb of 1/2-turns): 4 - +

Revolution Radius: 22.200

DNA routing parameters

Nb of helices per section: 16 - + Suggested: about 16

Nb of spirals: 2 - +

Winding parameter: -10 - +

Target total length: 7560

Figure S72: Design parameters of torus T4 in ENSnano.



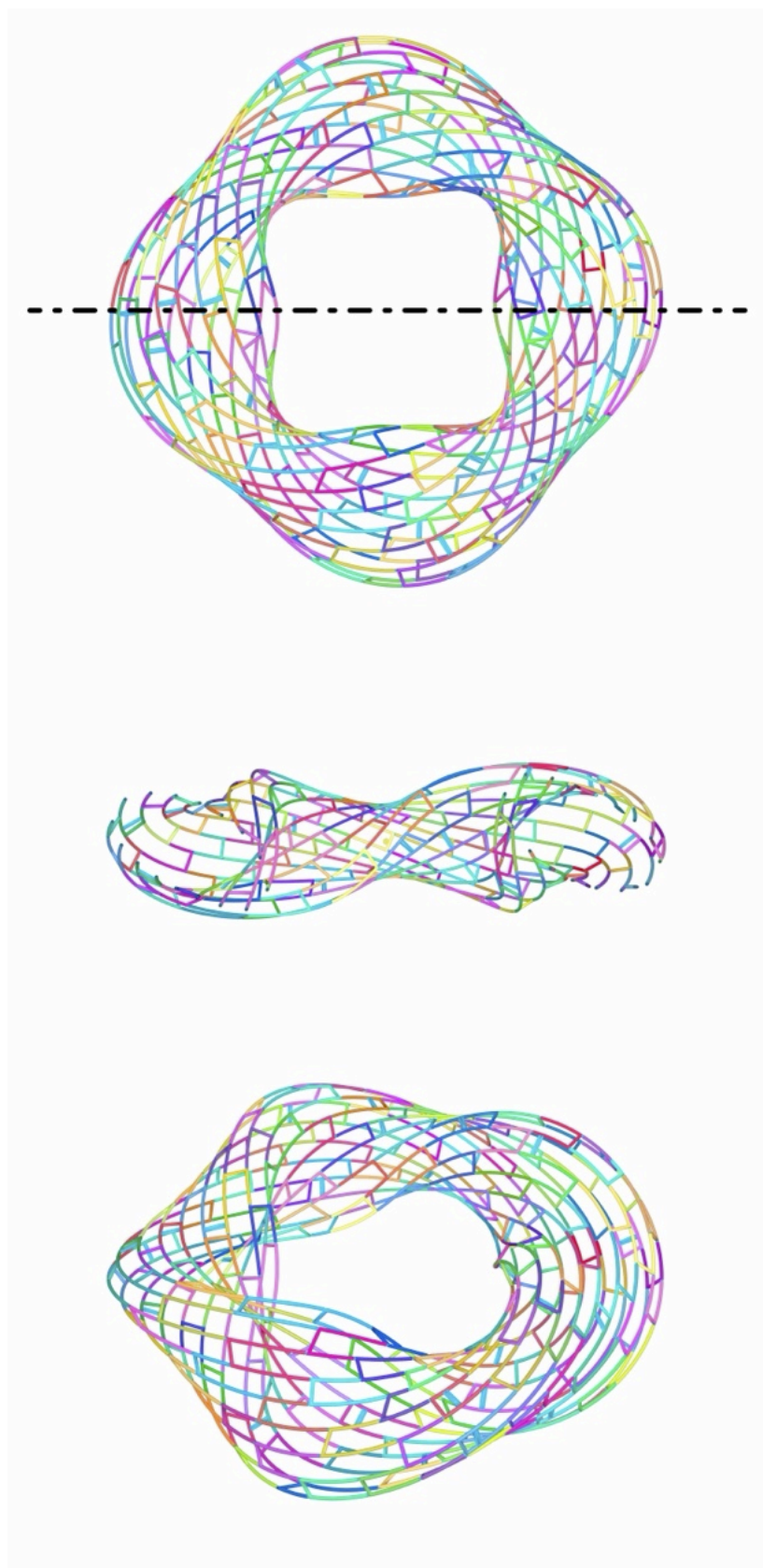


Figure S73: 3D view of the T4 design (line representation). From top to bottom: top view, cross-sectional view (along the dashed line indicated in the top view), and three-quarter view. For clarity, the scaffold strand is rendered with a slightly thinner line, leaving only its crossover pattern visible.

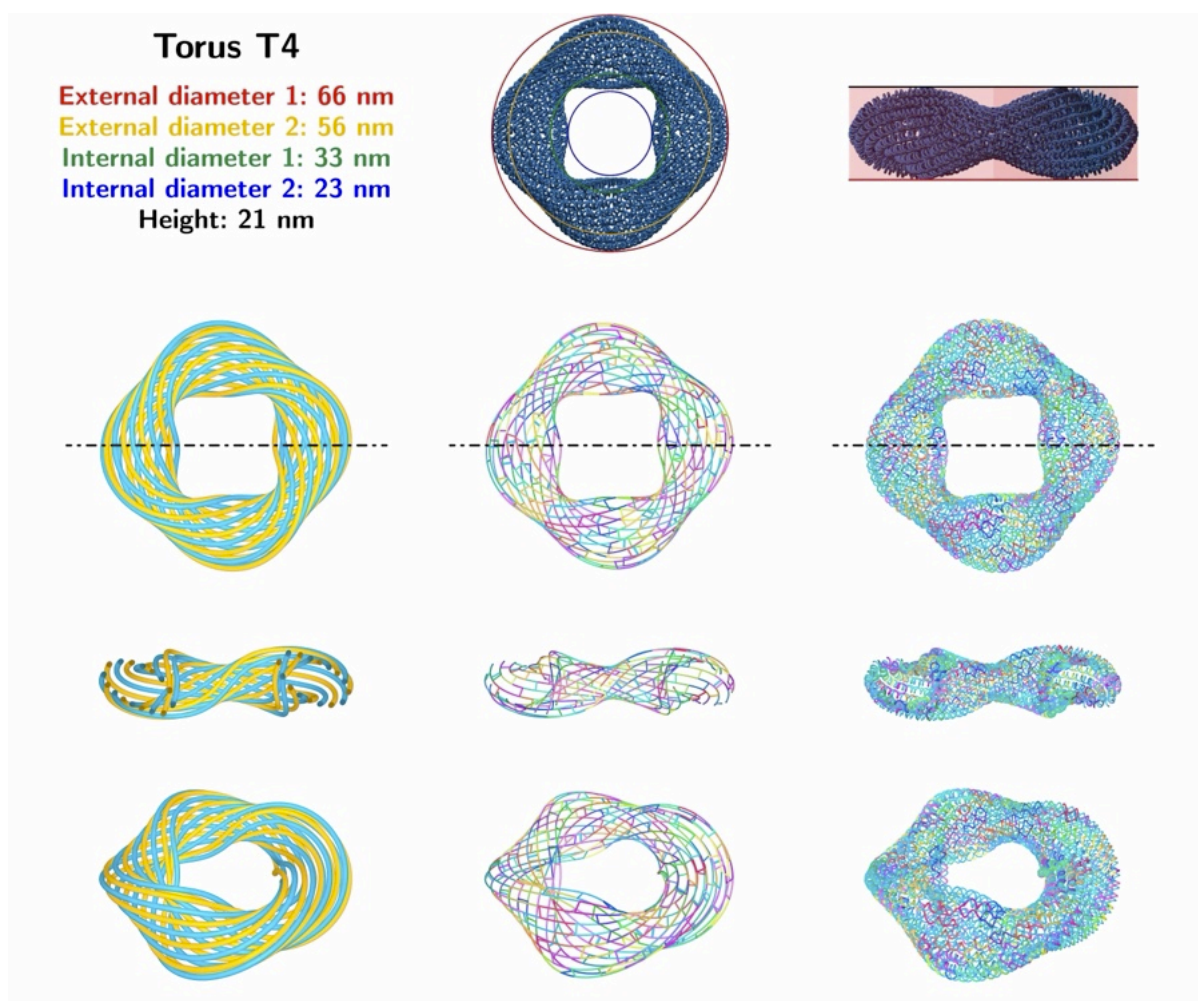


Figure S74: 3D views of the T4 design (helix, line, and full-strand representations). (Top row) Dimensions of the T4 design. The two external and two internal diameters are 66 nm (red), 56 nm (yellow), 33 nm (green) and 23 nm (blue), respectively, and the height is 21 nm. (Other rows) From top to bottom: top view, cross-sectional view (along the dashed line indicated in the top view), and three-quarter view. From left to right: helix, line, and full-strand representations.

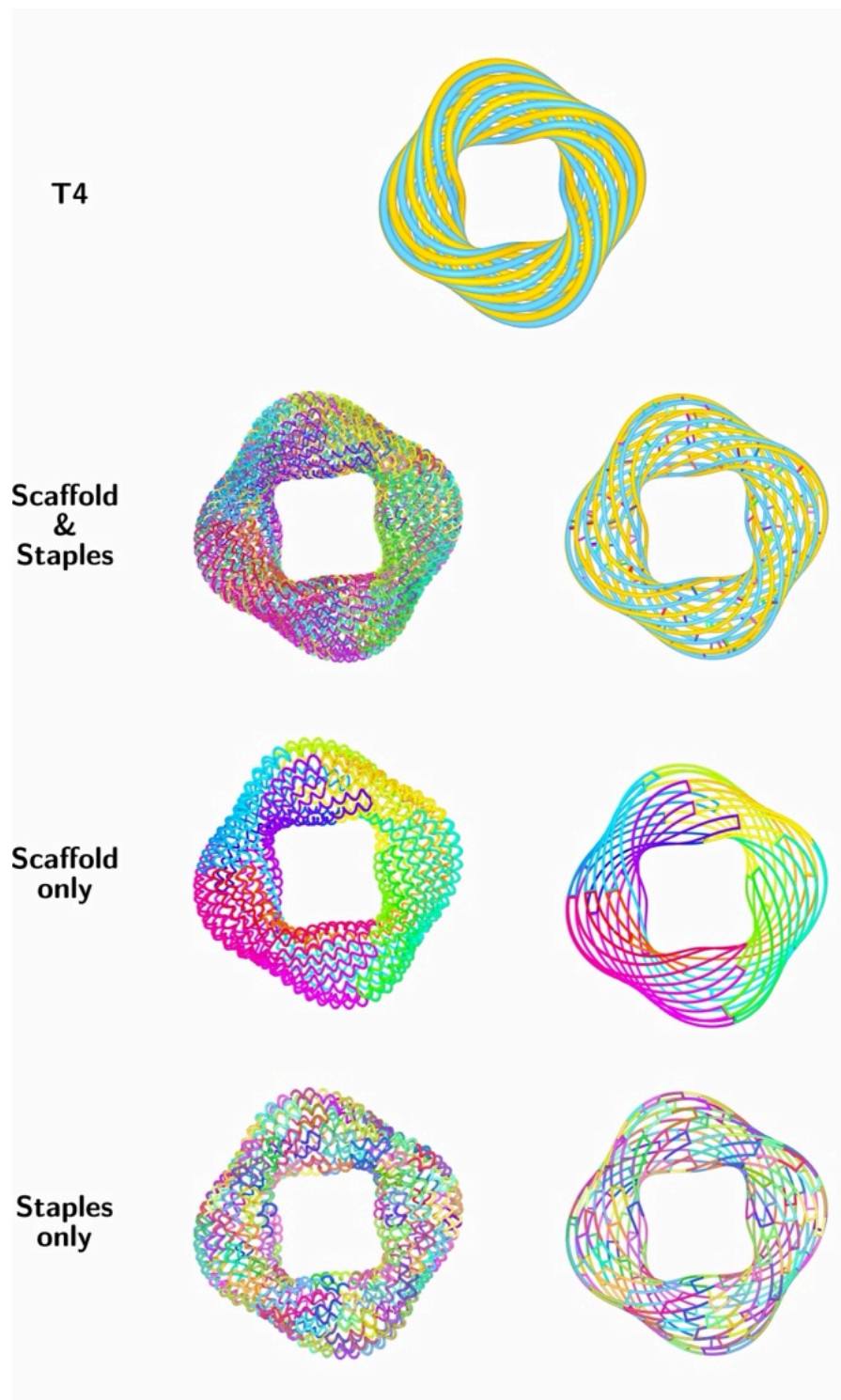


Figure S75: 3D view of the T4 design, helix (left) and line (right) representations. Views of the two spirals (top), the scaffold and staples (middle up), the scaffold (middle down) and the staples (bottom). Transversal bars on the line representation represent a crossover.

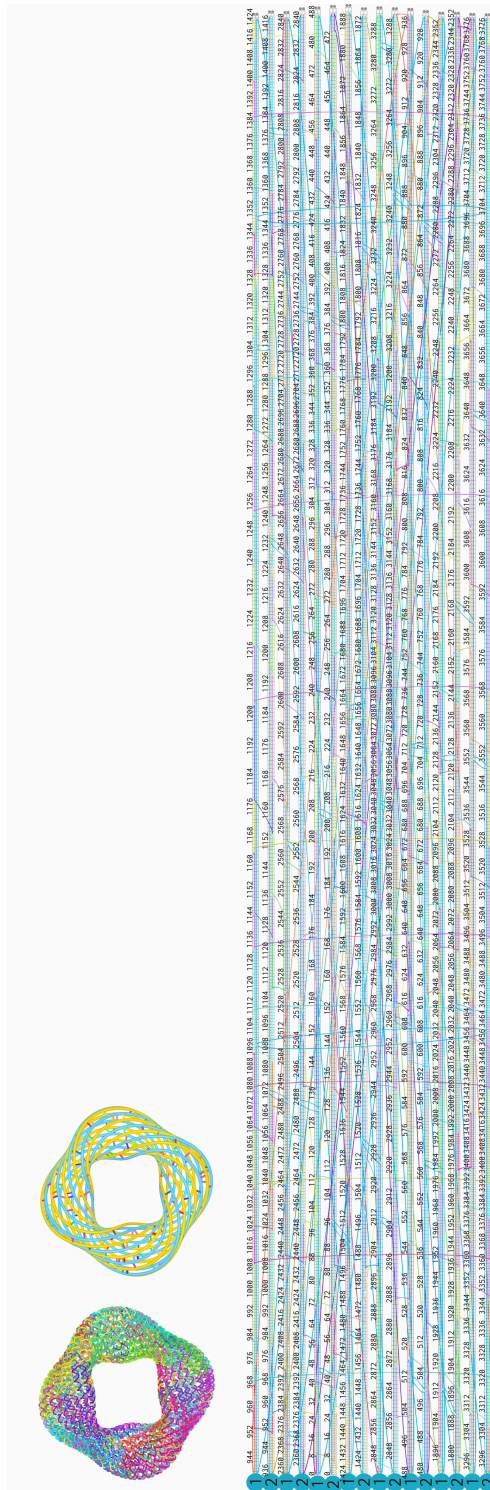


Figure S76: 2D view of the Torus T4 design. Scaffold and staples.



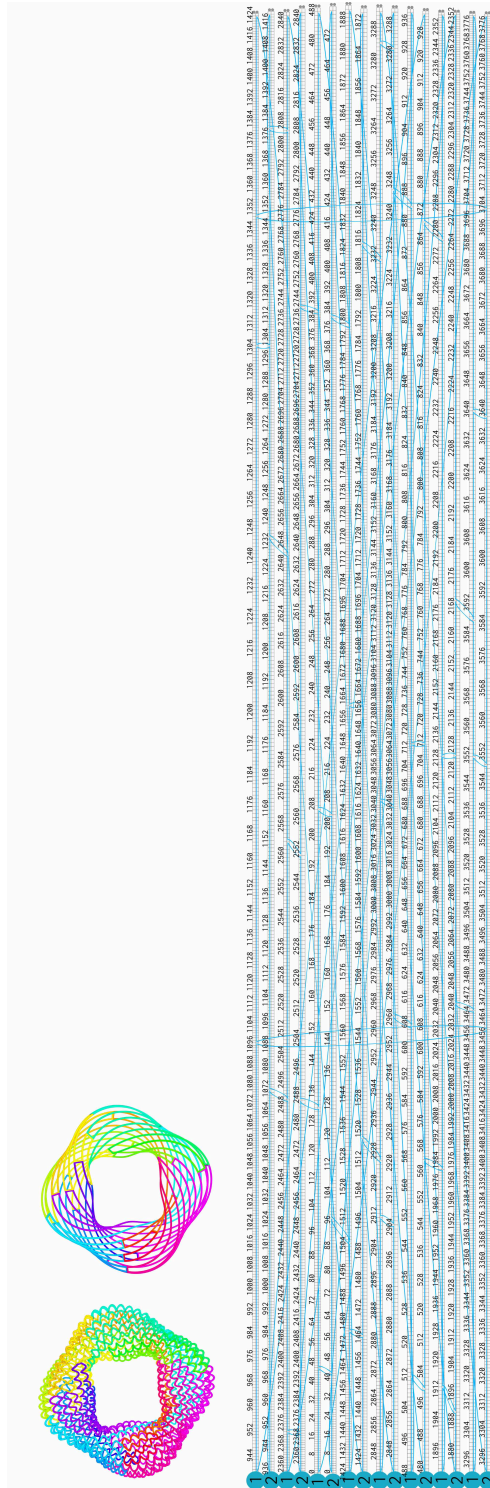


Figure S77: 2D view of the Torus T4 design. Scaffold only.

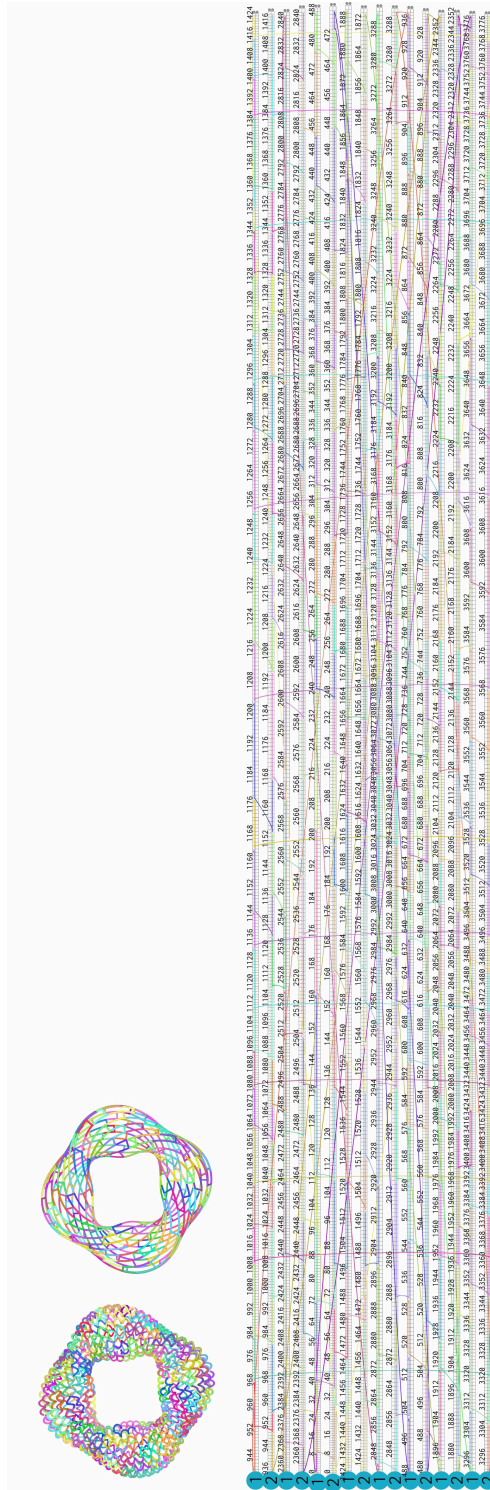


Figure S78: 2D view of the Torus T4 design. Staples only.

### S4.1.5 Torus T5 design

The twisted torus T2 is generated from a surface of revolution defined by a (1, 2) elliptical cross section and a  $3/2$  twist, with a helix routing comprising two helices that each make seven turns around the axis of revolution and a winding parameter of  $-11$ . The complete set of geometric and routing parameters is provided in fig. S79.

Revolution surface parameters

Curve type

Semi major axis

Semi minor axis

Twist (Nb of 1/2-turns): 5 - +

Revolution Radius

DNA routing parameters

Nb of helices per section: 14 - + Suggested: about 14

Nb of spirals: 2 - +

Winding parameter: -11 - +

Target total length

Figure S79: Design parameters of torus T5 in ENSnano.

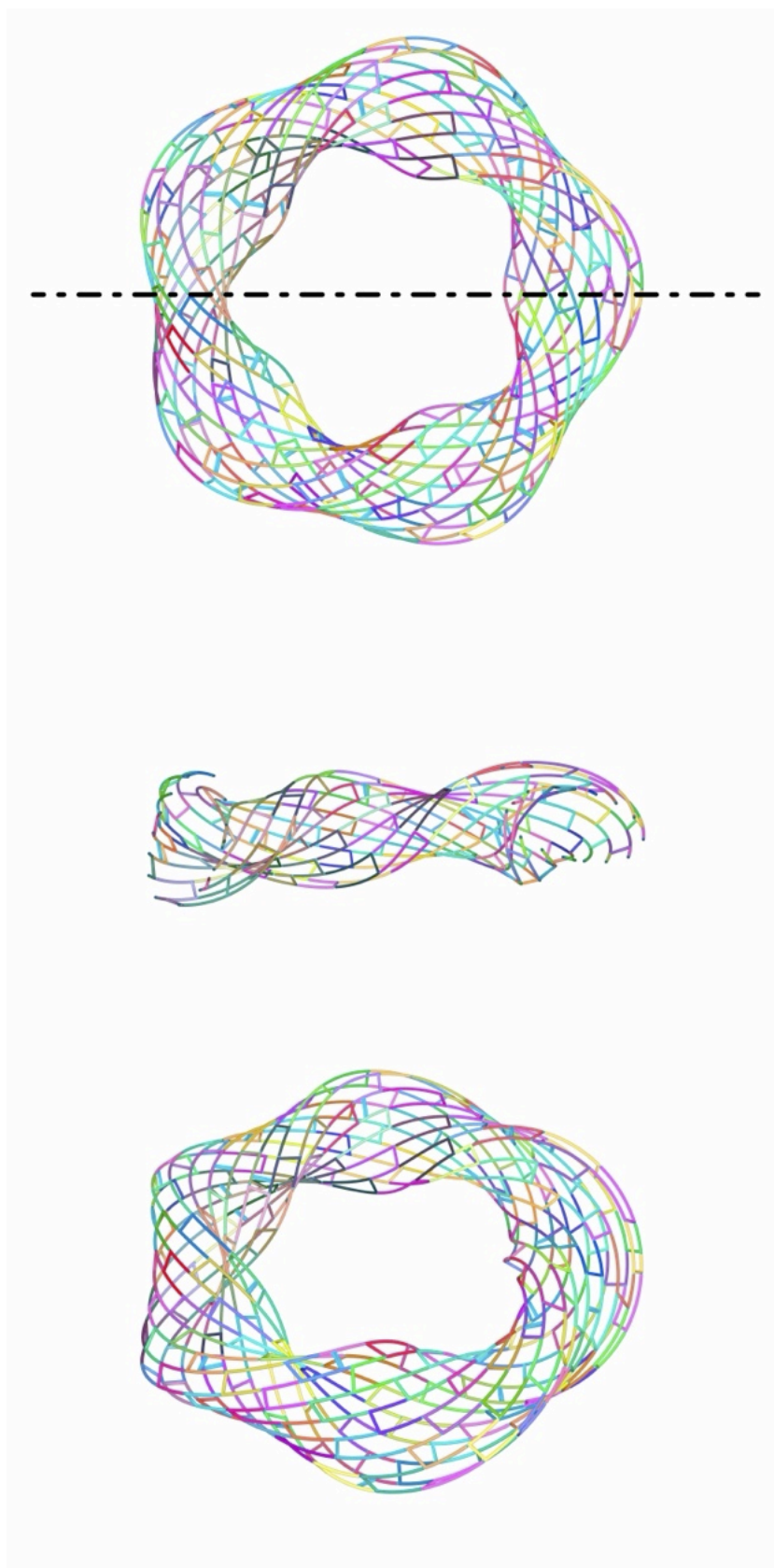


Figure S80: 3D view of the T5 design (line representation). From top to bottom: top view, cross-sectional view (along the dashed line indicated in the top view), and three-quarter view. For clarity, the scaffold strand is rendered with a slightly thinner line, leaving only its crossover pattern visible.



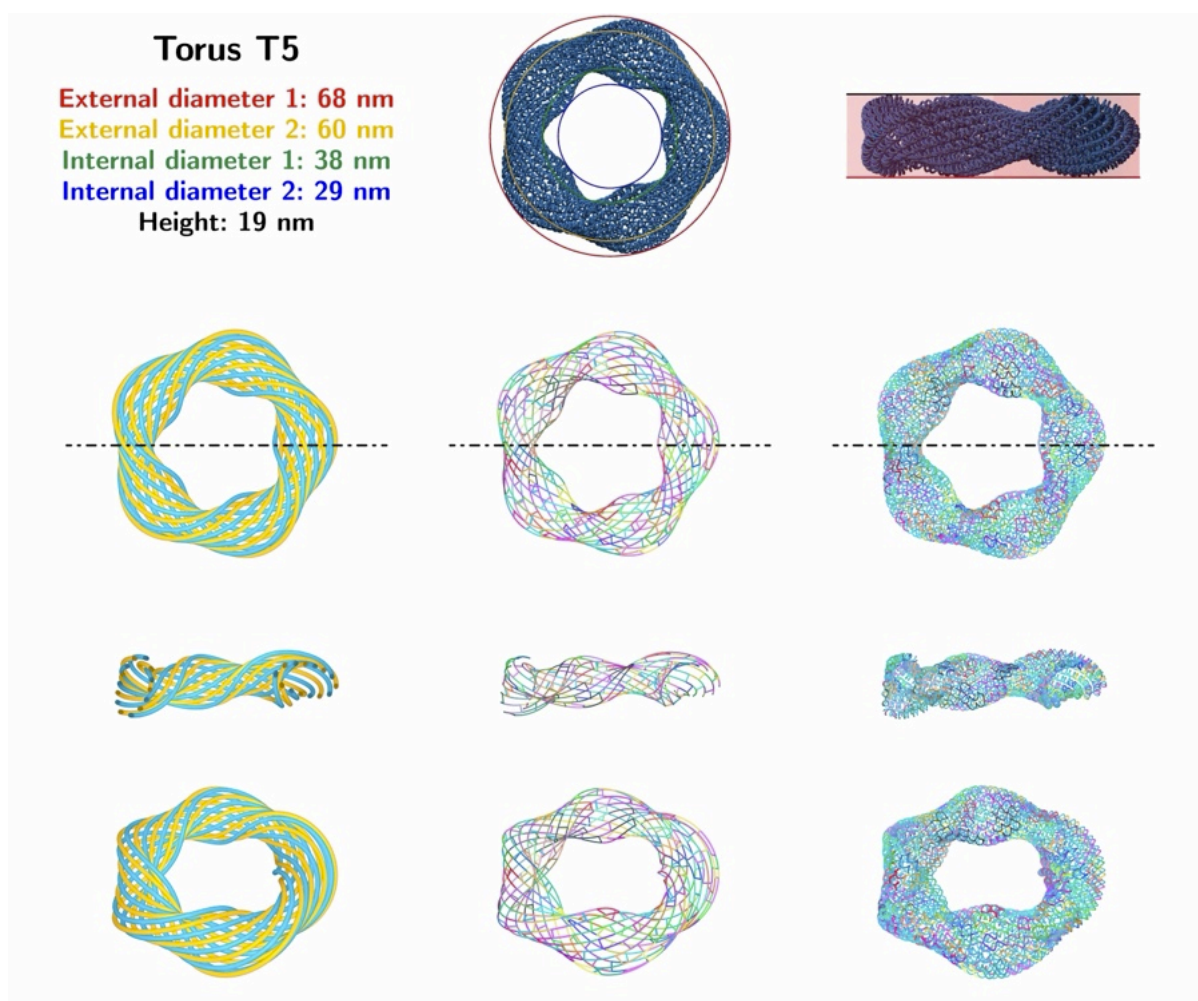


Figure S81: 3D views of the T5 design (helix, line, and full-strand representations). (Top row) Dimensions of the T5 design. The two external and two internal diameters are 68 nm (red), 60 nm (yellow), 38 nm (green) and 29 nm (blue), respectively, and the height is 19 nm. (Other rows) From top to bottom: top view, cross-sectional view (along the dashed line indicated in the top view), and three-quarter view. From left to right: helix, line, and full-strand representations.

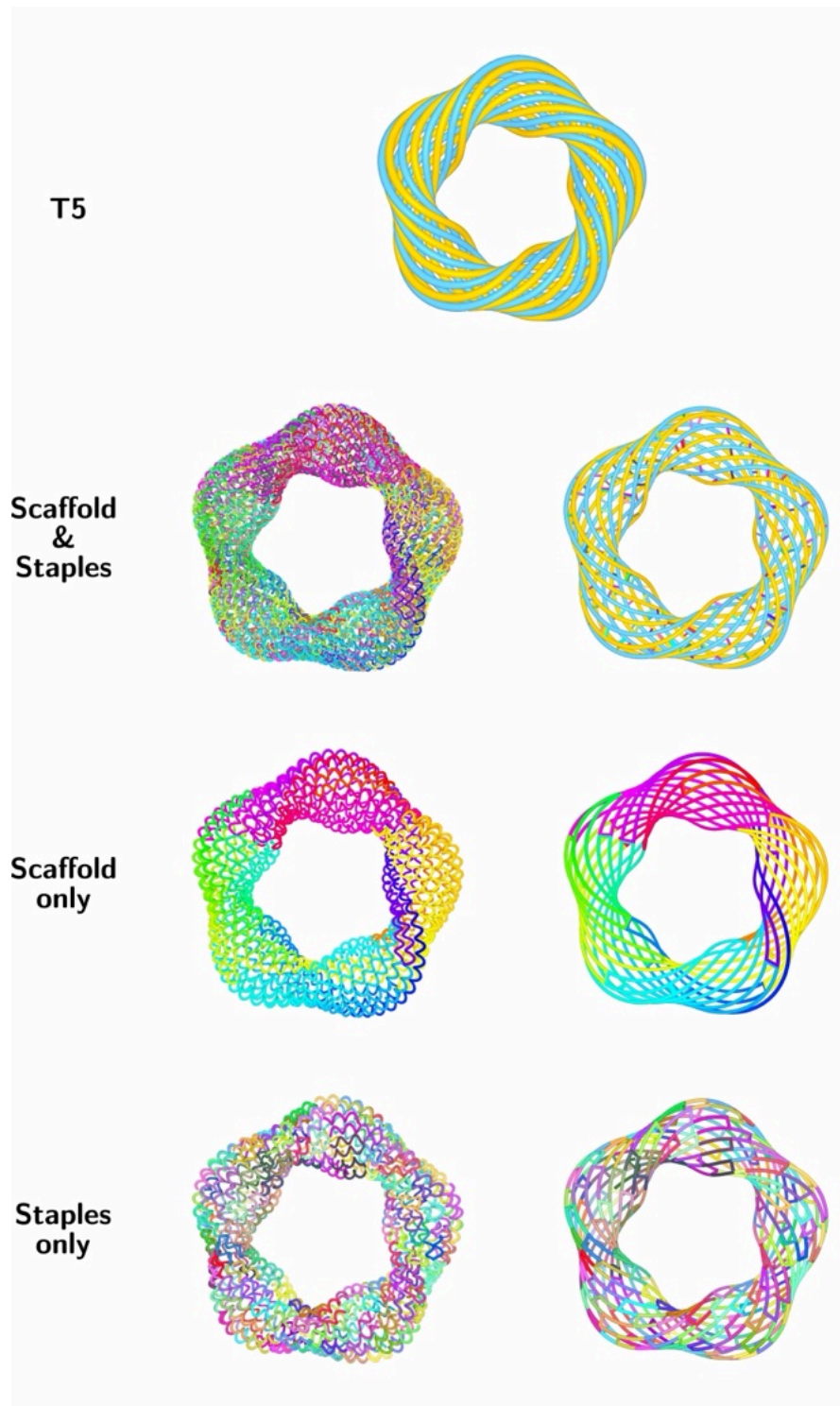


Figure S82: 3D views of the T5 design: helices, scaffold, and staples. (Top row) Helix representation. (Other rows) From top to bottom: all strands, scaffold strand only, and staple strands only. Left and right columns show the full-strand and line representations, respectively.

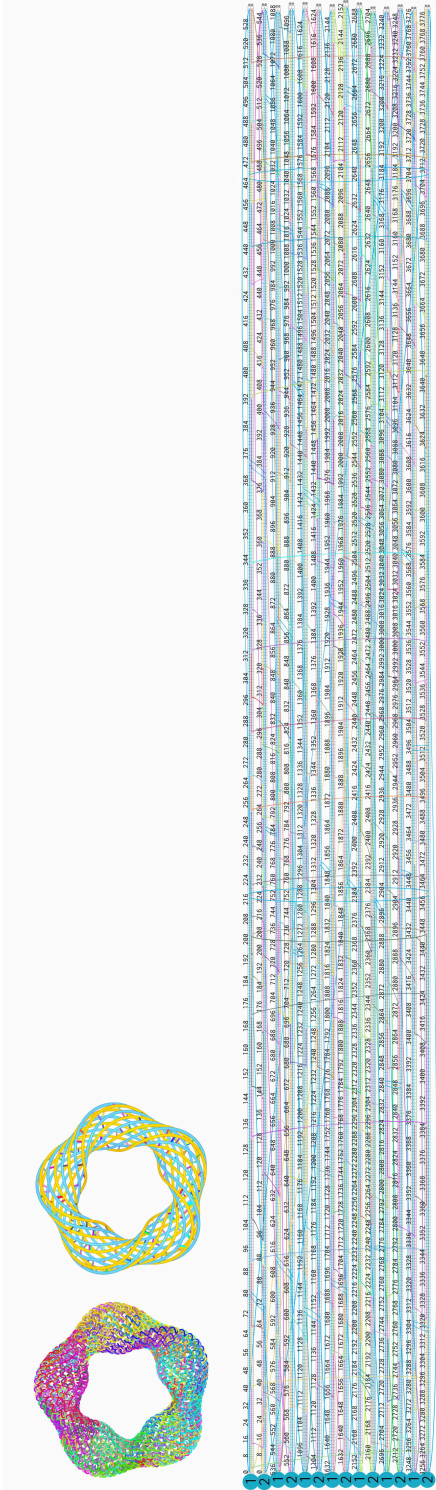


Figure S83: 2D view of the Torus T5 design. Scaffold and staples.

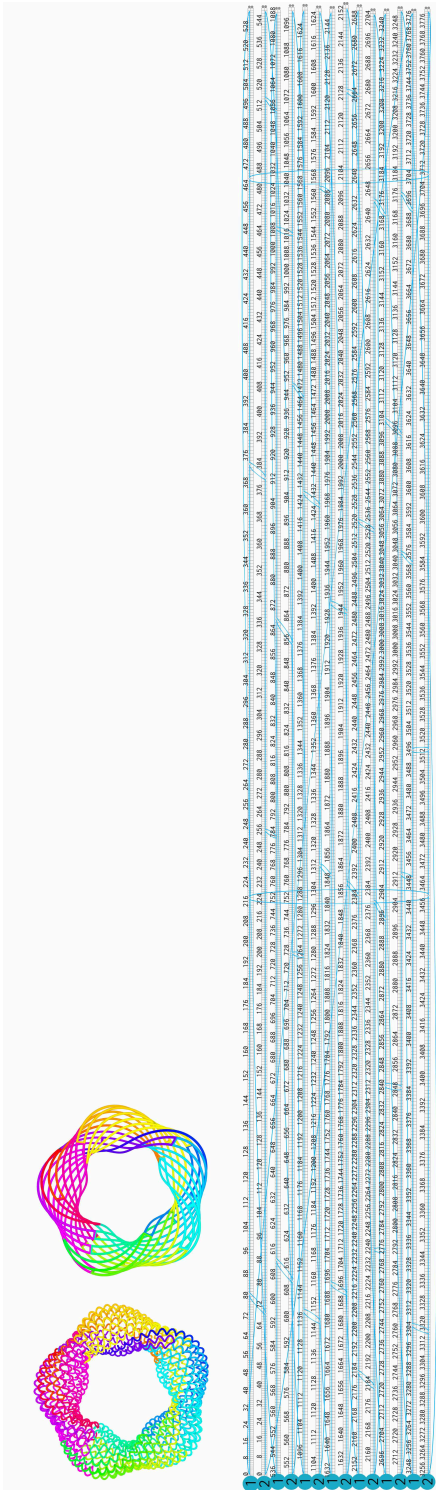


Figure S84: 2D view of the Torus T5 design. Scaffold only.



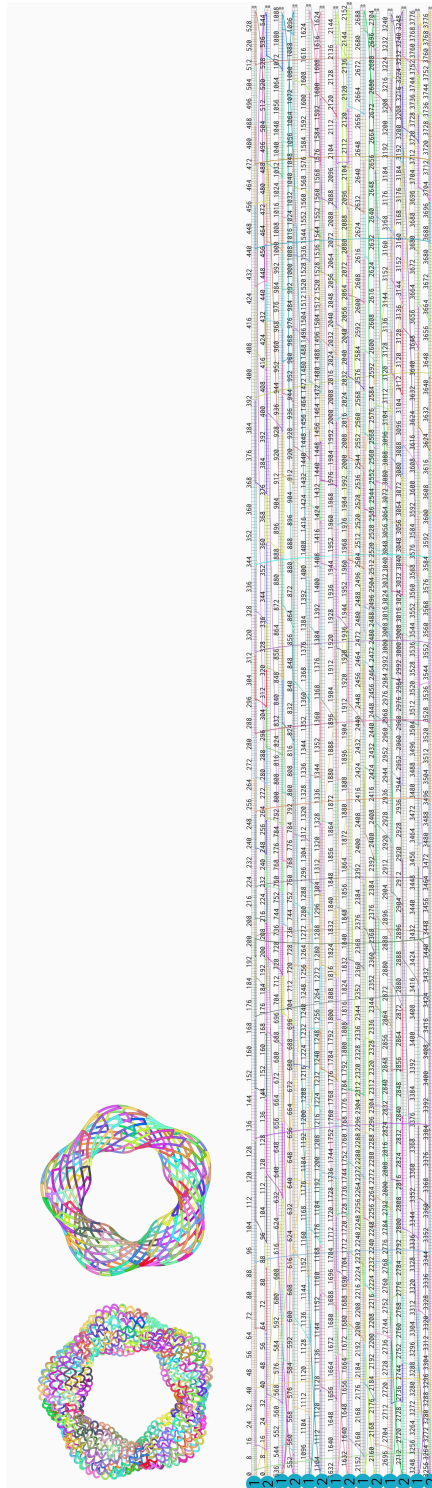


Figure S85: 2D view of the Torus T5 design. Staples only.

### S4.1.6 Torus T6 design

The twisted torus T6 is generated from a surface of revolution defined by a (1, 2) elliptical cross section and a  $3/2$  twist, with a helix routing comprising two helices that each make seven turns around the axis of revolution and a winding parameter of  $-12$ . The complete set of geometric and routing parameters is provided in fig. S86.

Revolution surface parameters

Curve type:

Semi major axis:

Semi minor axis:

Twist (Nb of 1/2-turns):  - +

Revolution Radius:

DNA routing parameters

Nb of helices per section:  - + Suggested: about 14

Nb of spirals:  - +

Winding parameter:  - +

Target total length:

Figure S86: Design parameters of torus T6 in ENSnano.

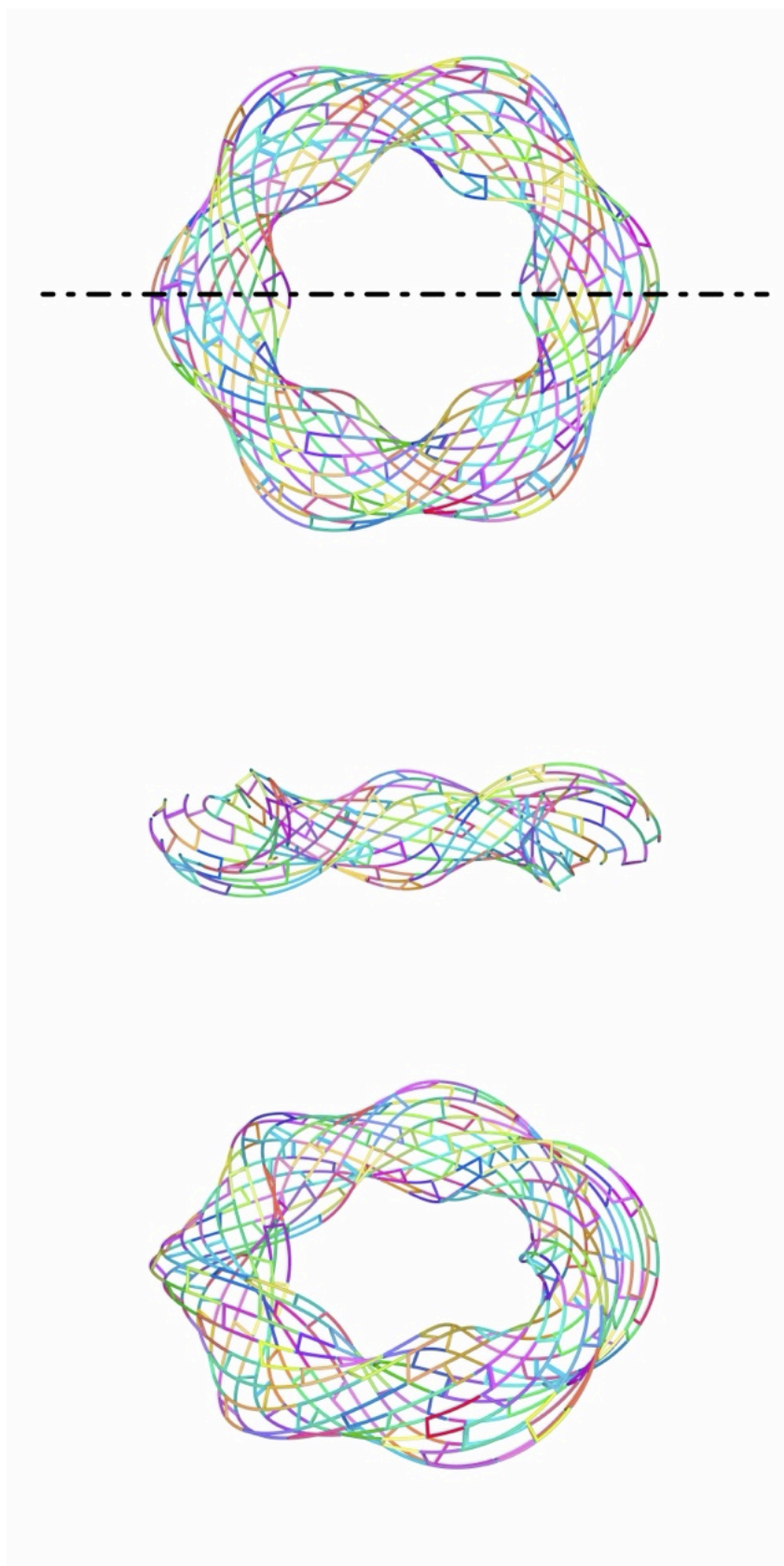


Figure S87: 3D view of the T6 design (line representation). From top to bottom: top view, cross-sectional view (along the dashed line indicated in the top view), and three-quarter view. For clarity, the scaffold strand is rendered with a slightly thinner line, leaving only its crossover pattern visible.

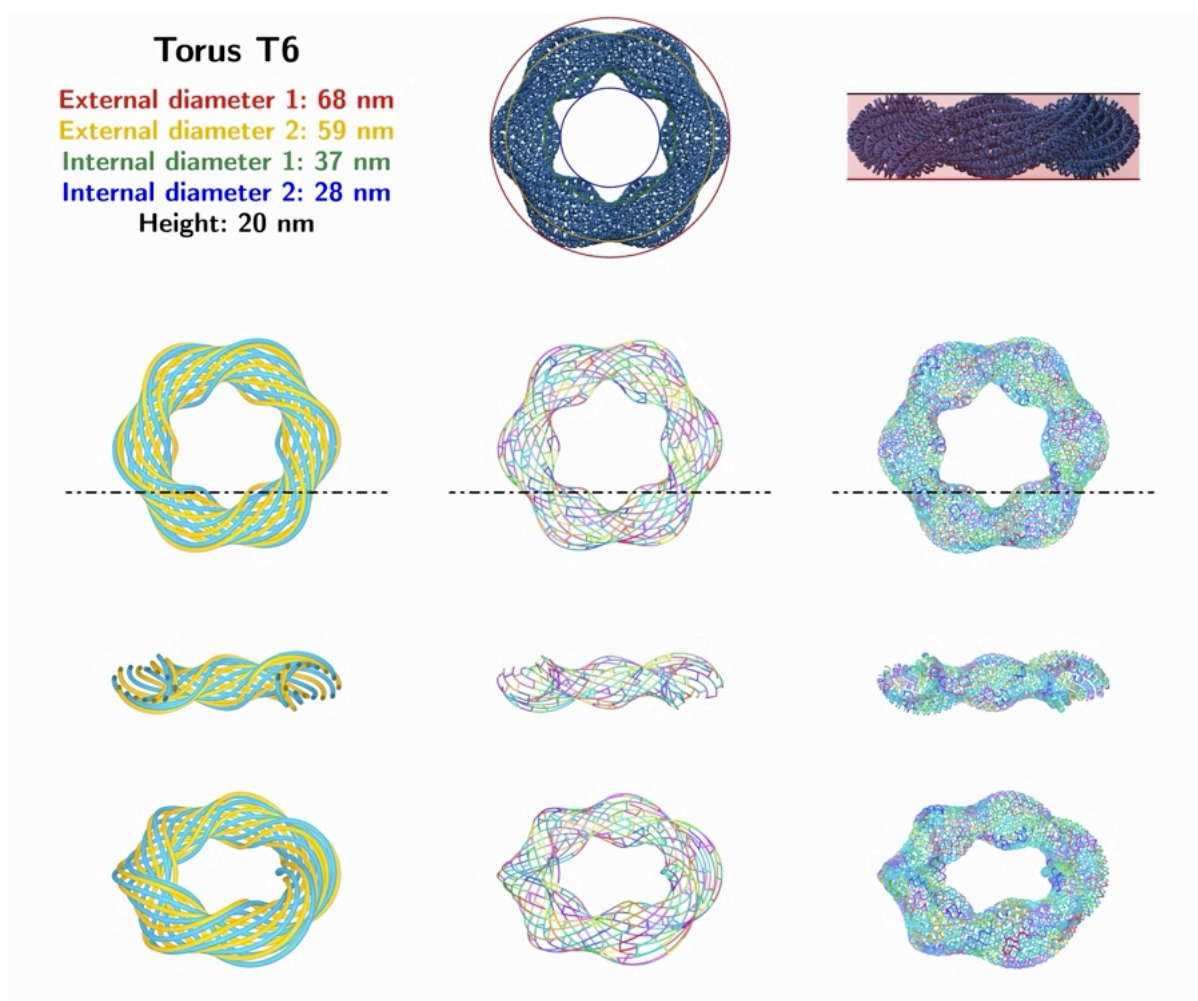


Figure S88: 3D views of the T6 design (helix, line, and full-strand representations). (Top row) Dimensions of the T6 design. The two external and two internal diameters are 68 nm (red), 59 nm (yellow), 37 nm (green) and 28 nm (blue), respectively, and the height is 20 nm. (Other rows) From top to bottom: top view, cross-sectional view (along the dashed line indicated in the top view), and three-quarter view. From left to right: helix, line, and full-strand representations.



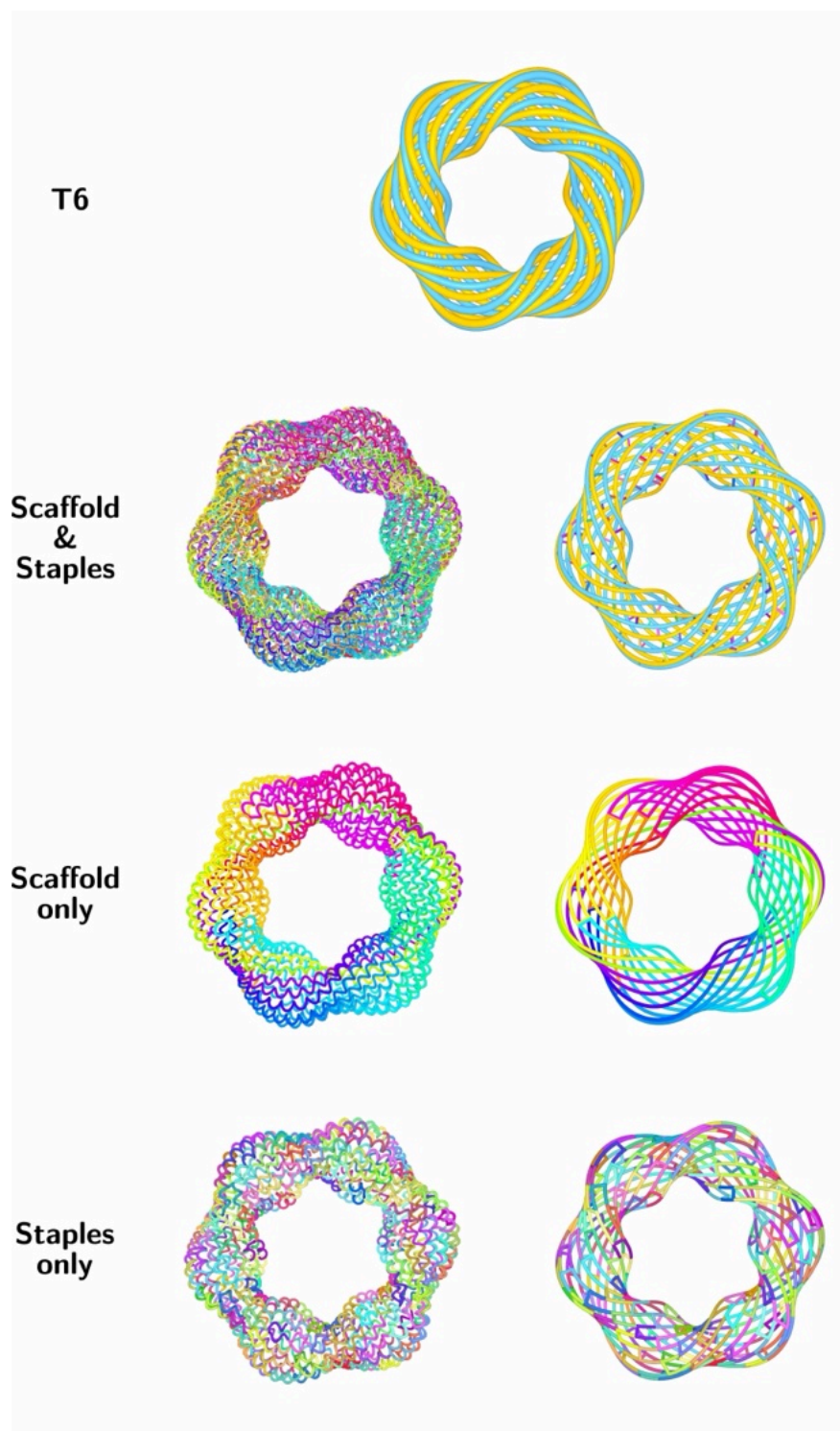


Figure S89: 3D views of the T6 design: helices, scaffold, and staples. (Top row) Helix representation. (Other rows) From top to bottom: all strands, scaffold strand only, and staple strands only. Left and right columns show the full-strand and line representations, respectively.



Figure S90: 2D view of the Torus T6 design. Scaffold and staples.

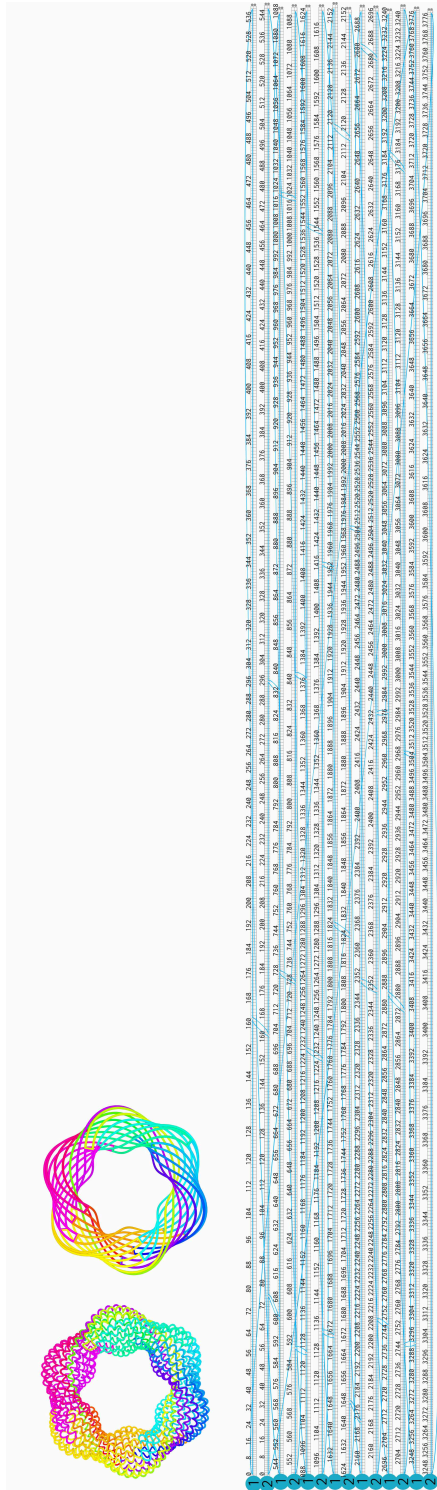


Figure S91: 2D view of the Torus T6 design. Scaffold only.

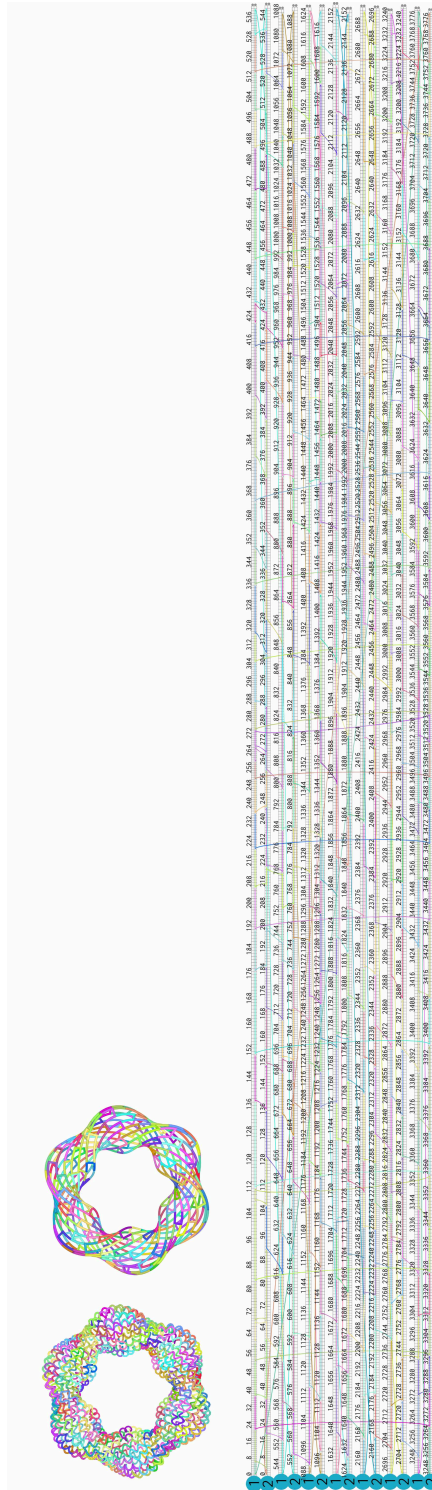


Figure S92: 2D view of the Torus T6 design. Staples only.



1387 **S4.2 Tori experimental validation**

1388 **S4.2.1 Torus T1 experimental validation**

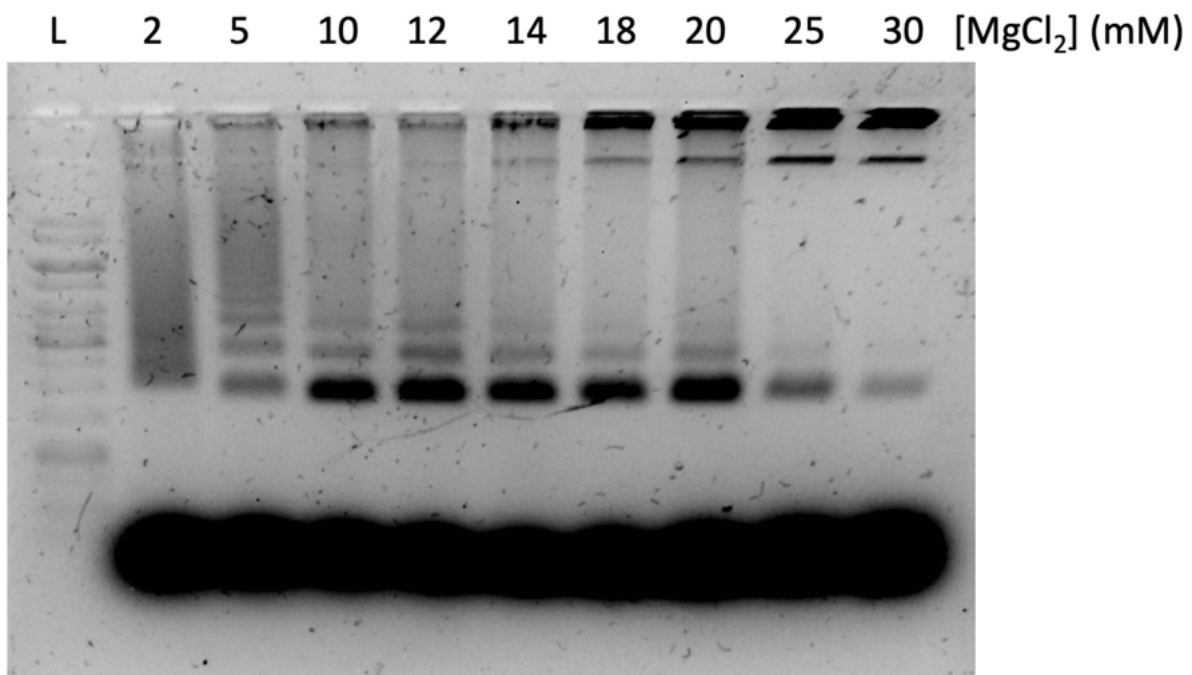


Figure S93: Gel analysis of the influence of MgCl<sub>2</sub> concentration on folding quality of the T1 origami. The foldings were analyzed by agarose gel electrophoresis (1%). L : 1kb DNA ladder ; Folding ramp: 65°C for 15 min, 60°C to 40°C -1°C/2h, 10°C Hold.

### S4.2.2 Tore T6 experimental optimization and validation

To determine good self-assembly conditions, we first focused on optimizing nanostructure T6. Agarose gel electrophoresis analysis of the assembly conditions revealed an optimal assembly at 18 mM with an annealing ramp over 33 hours (figs. S94 and S95). ns-TEM analysis of the gel purified T6 origami and two-dimensional class averages confirmed the fully formed origami according to the design and 2D projection simulations (figs. S96 and S97). We validated the accuracy of the designed structure by generating a 3D electron density map through single-particle cryo-TEM (Fig. 2 and figs. S98 and S99) which revealed the distinctive six twists per ring features within the resolution obtained.

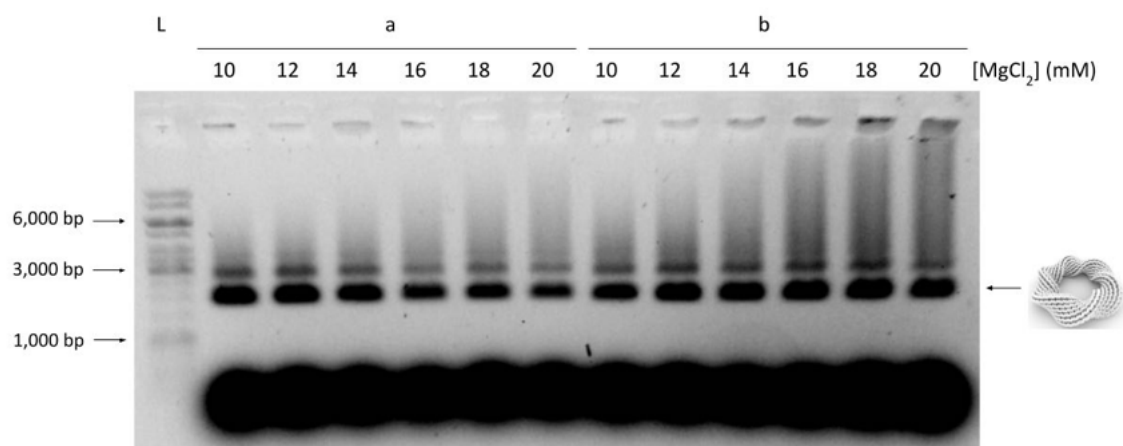


Figure S94: Gel analysis of the influence of  $\text{MgCl}_2$  concentration on folding quality of the T6. The foldings were analyzed by agarose gel electrophoresis (1%). L: 1kb DNA ladder; a: 65°C for 15 min, 60°C to 40°C -1°C/10minutes, 10°C Hold; b: 65°C for 15 min, 60°C to 40°C -1°C/1h, 10°C Hold.

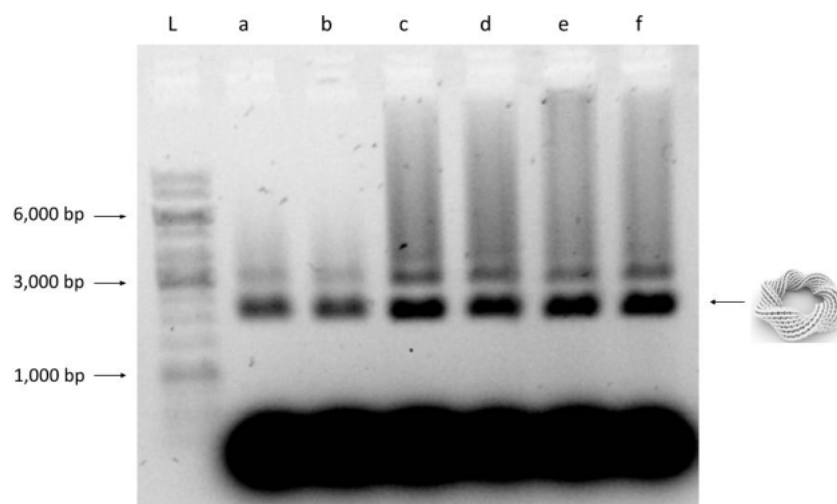


Figure S95: Gel analysis of the influence of different folding ramps on folding quality of the T6. The foldings were analyzed by agarose gel electrophoresis (1%). L: 1kb DNA ladder; a: 65°C for 15 min, 60°C to 40°C -1°C/5minutes, 10°C Hold; b: 65°C for 15 min, 60°C to 40°C -1°C/10minutes, 10°C Hold; c: 65°C for 15 min, 65°C to 55°C -1°C/10minutes, 55°C to 25°C -1°C/1h, 25°C Hold; d: 65°C for 15 min, 65°C to 50°C -1°C/10minutes, 50°C to 25°C -1°C/1h, 25°C Hold; e: 80°C for 15 min, 80°C to 55°C -1°C/10minutes, 55°C to 25°C -1°C/1h, 25°C Hold; f: 80°C for 15 min, 80°C to 25°C -1°C/10minutes, 25°C Hold.

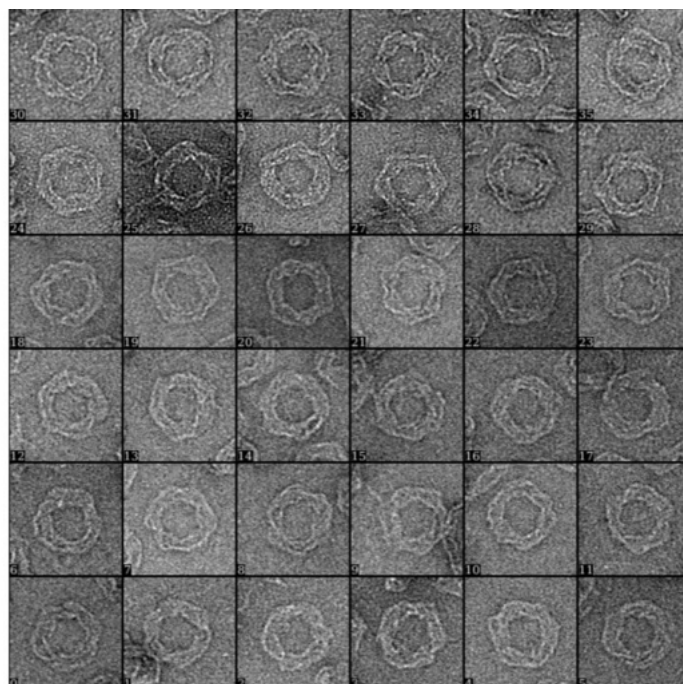


Figure S96: Transmission Electron Microscope (TEM) micrographs of typical particles of the T6. Each box is 99 nm x 99 nm.



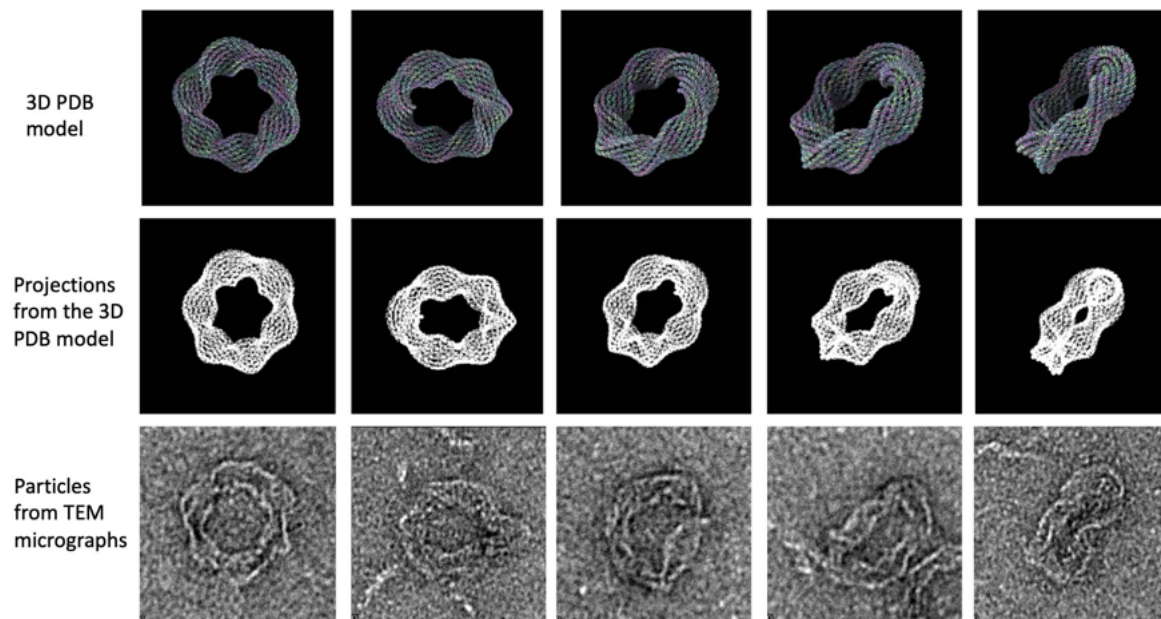


Figure S97: T6 projections from 3D PDB model. Observed particles and representative projections from 3D PDB model of the T6 showing different orientations from TEM micrographs. Each box is 99 nm x 99 nm.

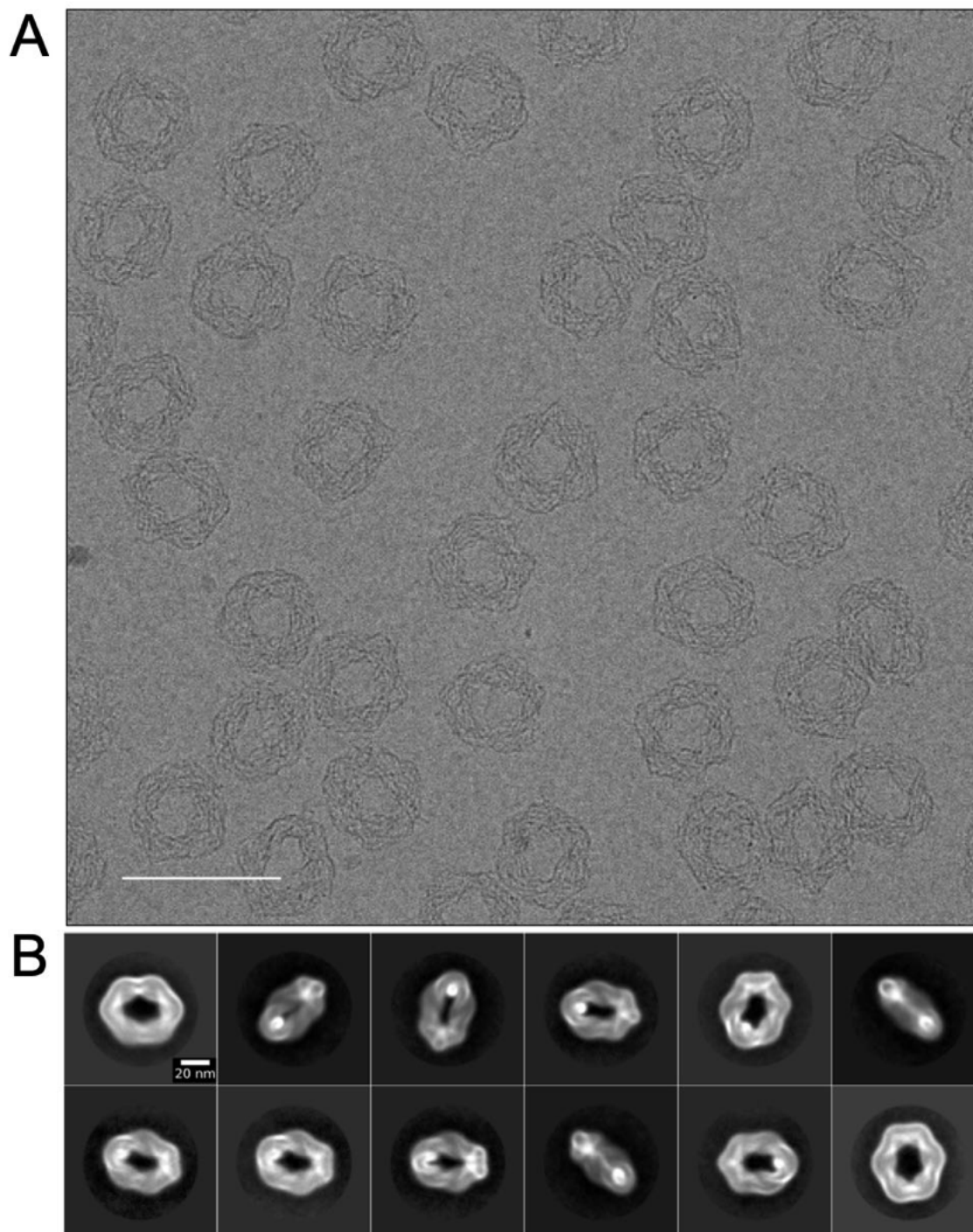


Figure S98: Cryo-EM images analysis for T6. A: Exemplary micrograph of a total of 15,127 movie stacks. Scale bar = 100 nm. B: Representative 2D Class averages.

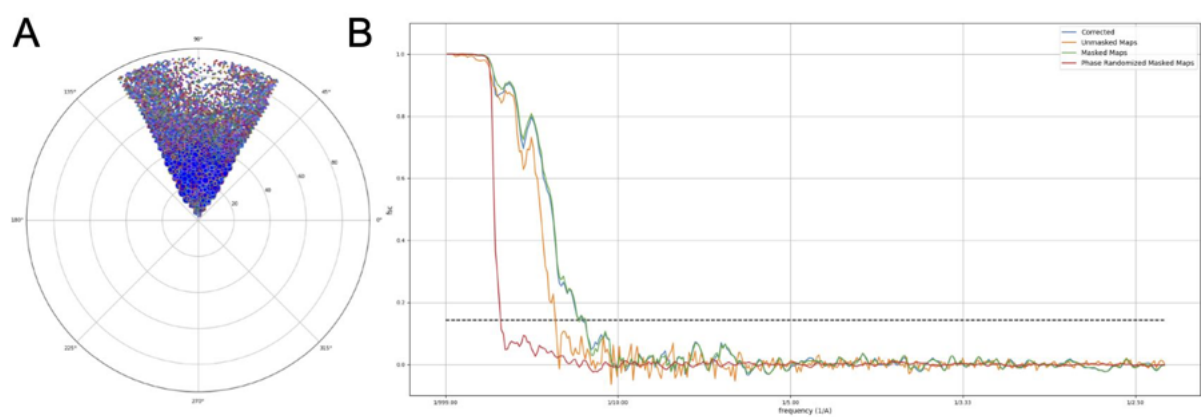


Figure S99: Cryo-EM map determination for T6. A: Histogram representing the angular distribution of particles. B: FSC plot.

### S4.2.3 Tori T2 to T4 experimental validation

This optimized self-assembly protocol was employed for three additional structures (T2 to T4) (figs. S100 to S102 and had to be optimized for T5 (figs. S103 to S106).

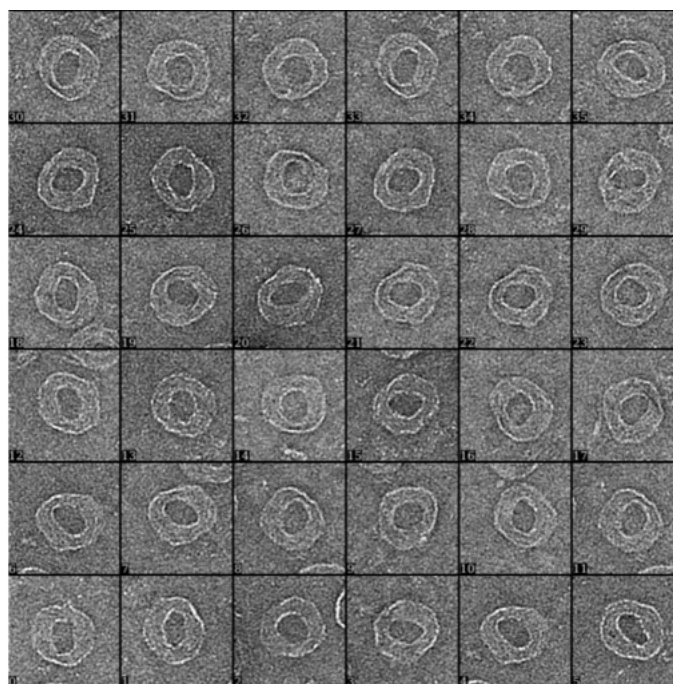


Figure S100: Transmission Electron Microscope (TEM) micrographs of typical particles of the T2. Each box is 99 nm x 99 nm.

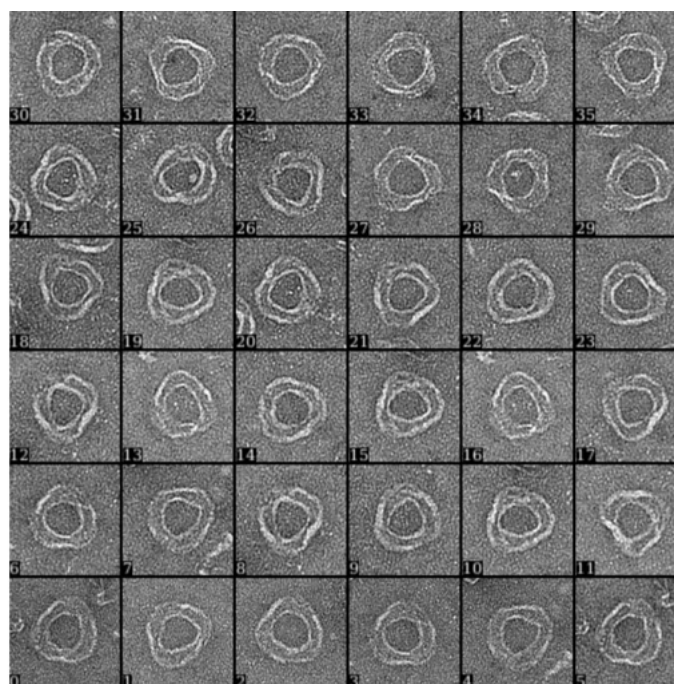


Figure S101: Transmission Electron Microscope (TEM) micrographs of typical particles of the T3. Each box is 99 nm x 99 nm.



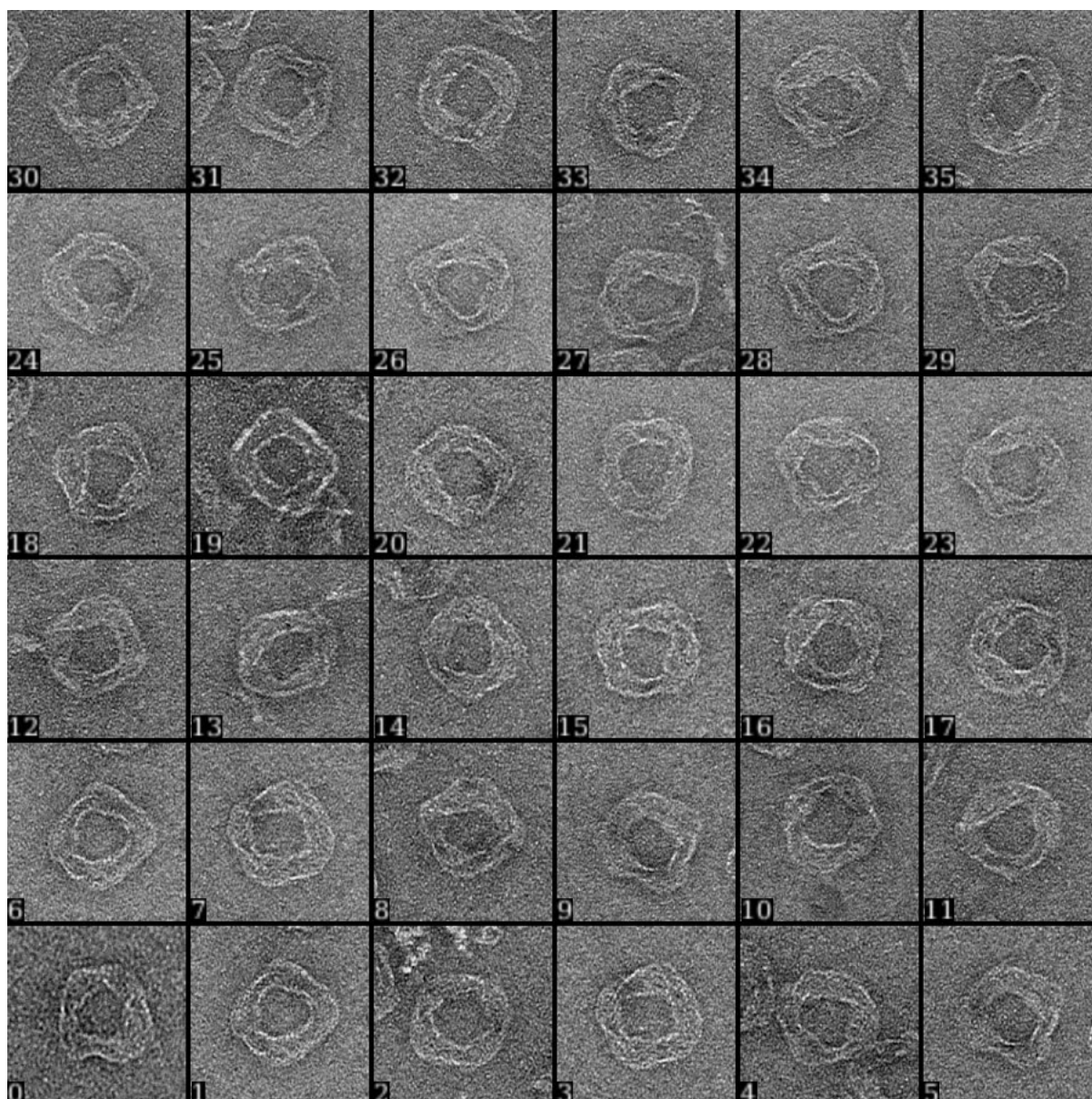


Figure S102: Transmission Electron Microscope (TEM) micrographs of typical particles of the T4. Each box is 99 nm x 99 nm.

#### S4.2.4 T5: optimization of folding conditions and experimental validation

**Optimization of the folding conditions of the T5 origami (figs. S103 to S106).** Despite four different tested folding ramps with a scaffold concentration of 20 nM and staples oligonucleotides in a 10-fold excess, the gel analysis of the T5 showed some aggregates in the well, with different  $\text{MgCl}_2$  concentrations and folding ramps (figs. S103 to S106).

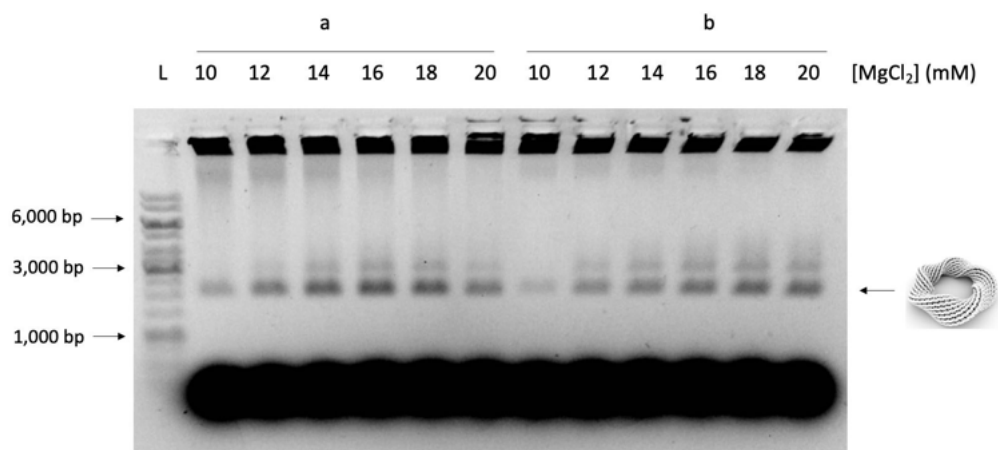


Figure S103: Gel analysis of the influence of  $\text{MgCl}_2$  concentration on folding quality of the T5. The foldings were analyzed by agarose gel electrophoresis (1%). L: 1kb DNA ladder ; a: 65°C for 15 min, 60°C to 40°C -1°C/10minutes, 10°C Hold ; b: 65°C for 15 min, 60°C to 40°C -1°C/1h, 10°C Hold.

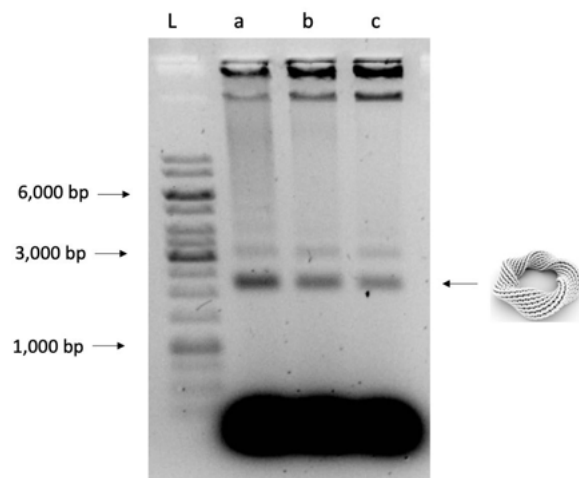


Figure S104: Gel analysis of the influence of the folding ramp on folding quality of the T5 with 18 MgCl<sub>2</sub>. The foldings were analyzed by agarose gel electrophoresis (1%). L: 1kb DNA ladder; a: 65°C for 15 min, 60°C to 40°C -1°C/2minutes, 10°C Hold; b: 65°C for 15 min, 60°C to 40°C -1°C/5minutes, 10°C Hold; c: 65°C for 15 min, 60°C to 40°C -1°C/10minutes, 10°C Hold.

1405 To improve the folding's yield, we tested different concentrations of scaffold and staples oligonu-  
 1406 cleotides. Agarose gel electrophoresis analysis showed that while we still had a well defined monomeric  
 1407 band with 5 nM of scaffold, we had less aggregates in the well, indicating a better yield of the origami at  
 1408 this scaffold concentration. These results were especially observed with a folding ramp over a 3.5 hours  
 1409 period rather than with one over 33 hours (fig. S105).

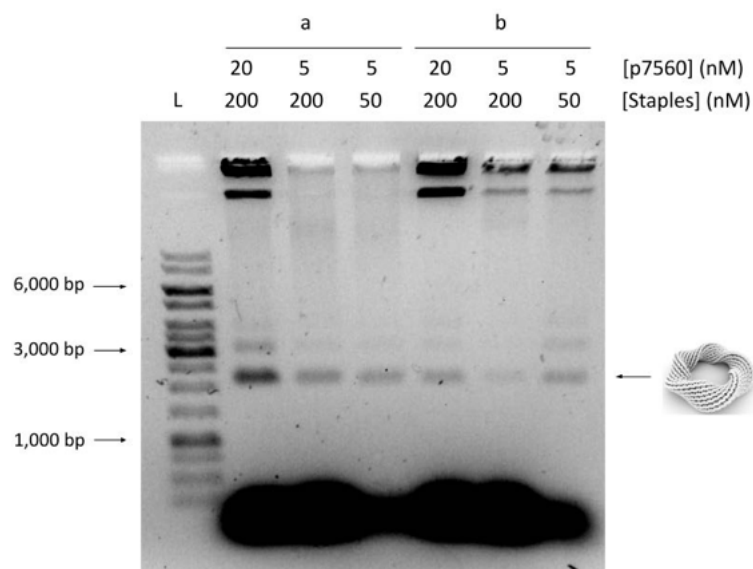


Figure S105: Gel analysis of the influence of scaffold M13 7560 and staples concentration on folding quality of the T5. The foldings were analyzed by agarose gel electrophoresis (1%).. L: 1kb DNA ladder; a: 65°C for 15 min, 60°C to 40°C -1°C/10minutes, 10°C Hold; b: 65°C for 15 min, 65°C to 55°C -1°C/10minutes, 55°C to 25°C -1°C/1h, 25°C Hold.

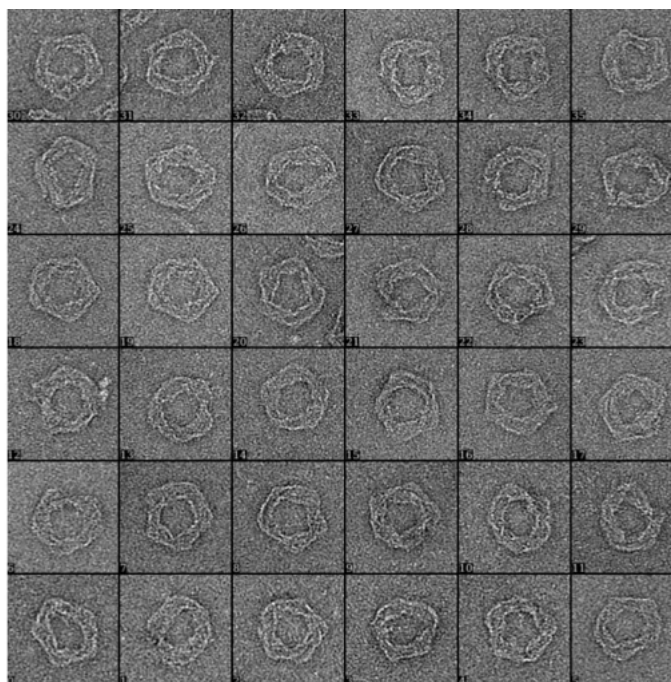


Figure S106: Transmission Electron Microscope (TEM) micrographs of typical particles of the T5. Each box is 99 nm x 99 nm.



## S5 Double-sphere, Figures S107 to S115

### S5.1 Double-sphere DS design

The double-sphere DS is generated from a surface of revolution defined by a C-shaped cross section without twist. The helix routing consists of two helices, each making fourteen turns around the axis of revolution, with a winding parameter of +2. The complete set of geometric and routing parameters is provided in fig. S107.

The image shows a software interface with a dark background and white text. It is divided into two main sections: 'Revolution surface parameters' and 'DNA routing parameters'.  
**Revolution surface parameters:**  
- 'Curve type' is a dropdown menu set to 'Two spheres'.  
- 'Radius extern' is a text input field with the value '25.000'.  
- 'Radius intern' is a text input field with the value '17.000'.  
- 'Radius tube' is a text input field with the value '7.600'.  
- 'Smooth cell' is a text input field with the value '0.040'.  
- 'Twist (Nb of turns):' is a control with a value of '0' and minus/plus buttons.  
- 'Revolution Radius' is a text input field with the value '-15.000'.  
**DNA routing parameters:**  
- 'Nb of helices per section:' is a control with a value of '34', minus/plus buttons, and a suggestion 'Suggested: about 28'.  
- 'Nb of spirals:' is a control with a value of '2' and minus/plus buttons.  
- 'Winding parameter:' is a control with a value of '2' and minus/plus buttons.  
- 'Target total length' is a text input field with the value '8064'.

Figure S107: Surface-of-revolution and helix-routing parameters of the double-sphere DS in ENSnano.

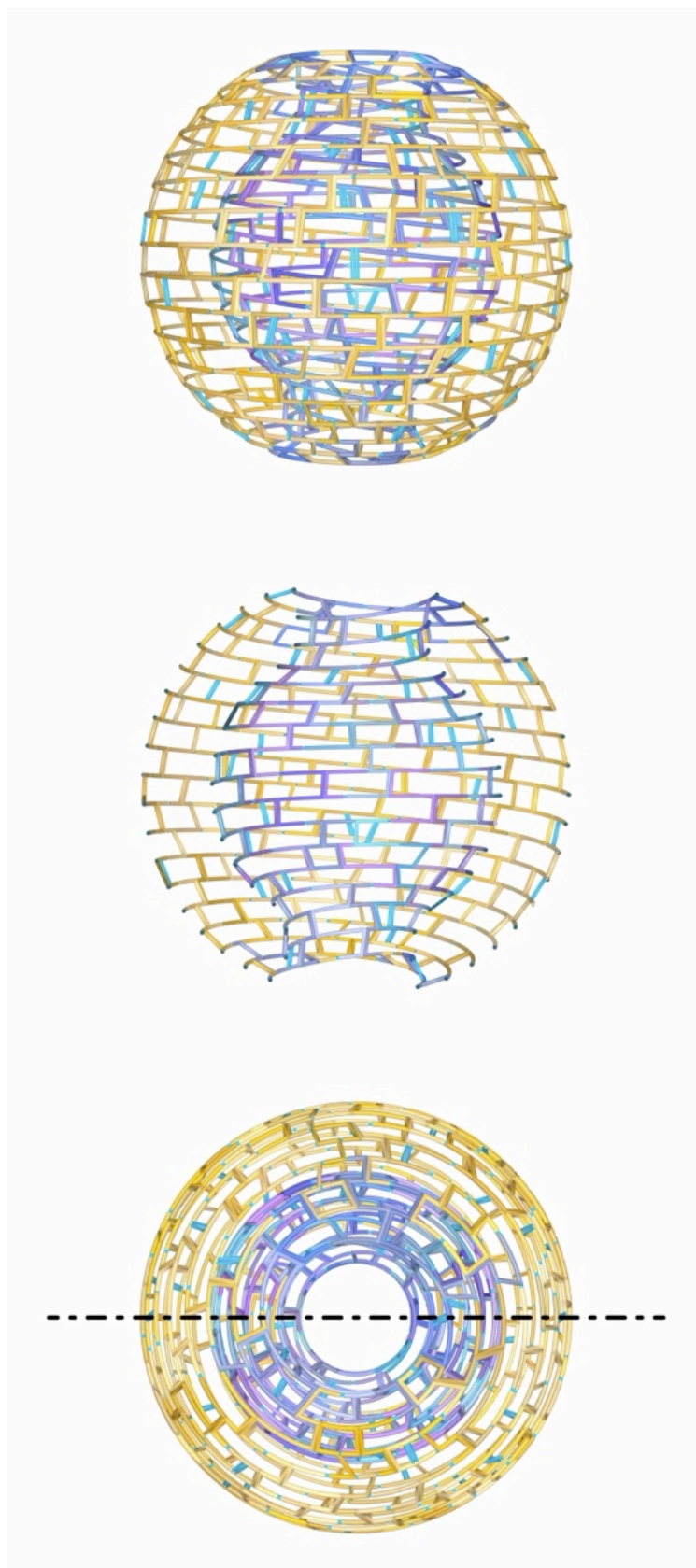


Figure S108: 3D view of the DS design (line representation). From bottom to top: top view, cross-sectional view (along the dashed line indicated in the top view), and side view. For clarity, the scaffold strand is rendered with a slightly thinner cylinder, leaving only its crossover pattern visible.

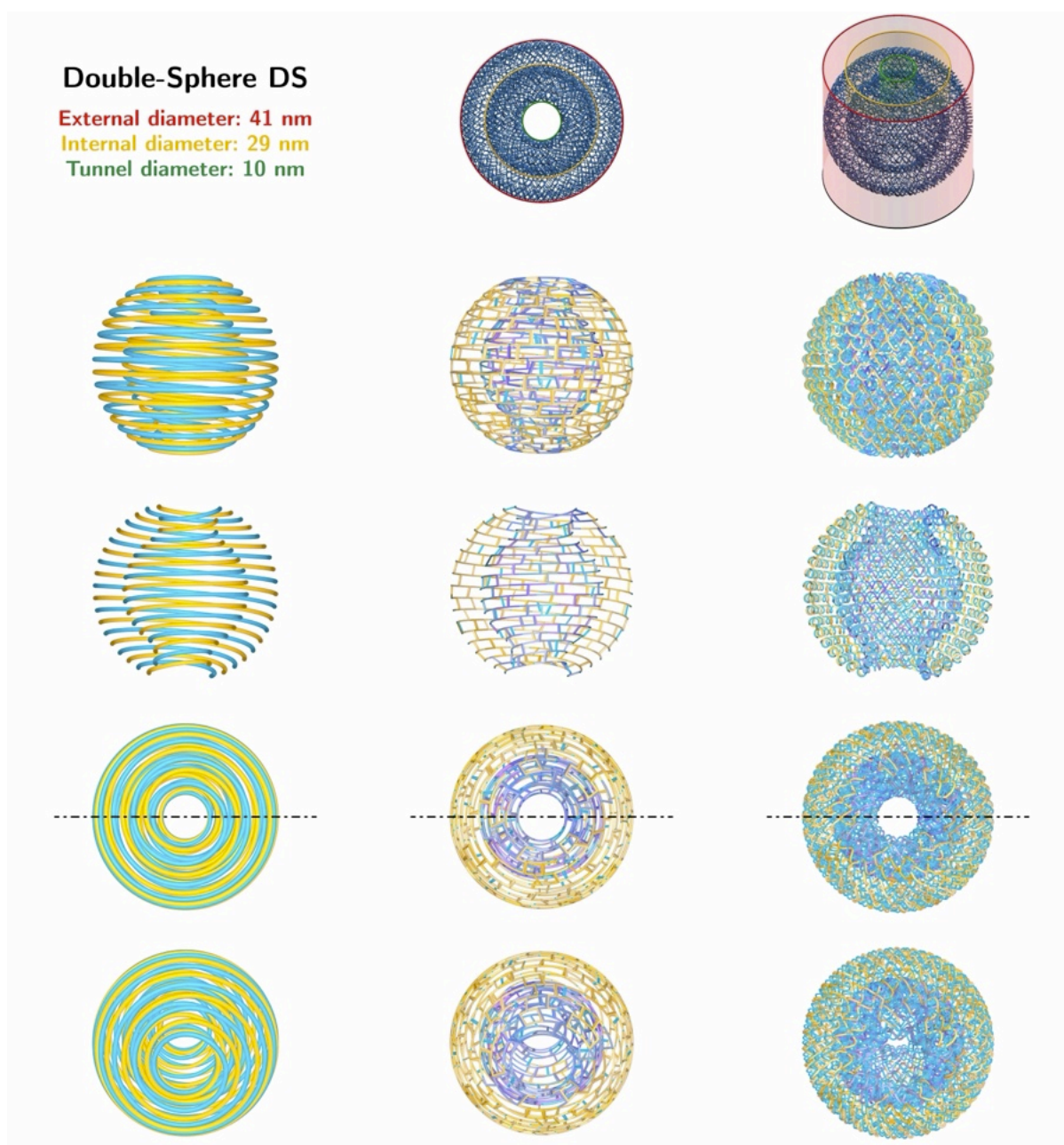


Figure S109: 3D views of the DS design (helix, line, and full-strand representations). (Top row) Dimensions of the DS design. The external and internal diameters are 41 nm (red) and 29 nm (yellow), respectively, and the tunnel diameter is 10 nm. (Other rows) From top to bottom: side view, cross-sectional view (along the dashed line indicated in the top view), top view and three-quarter view. From left to right: helix, line, and full-strand representations.



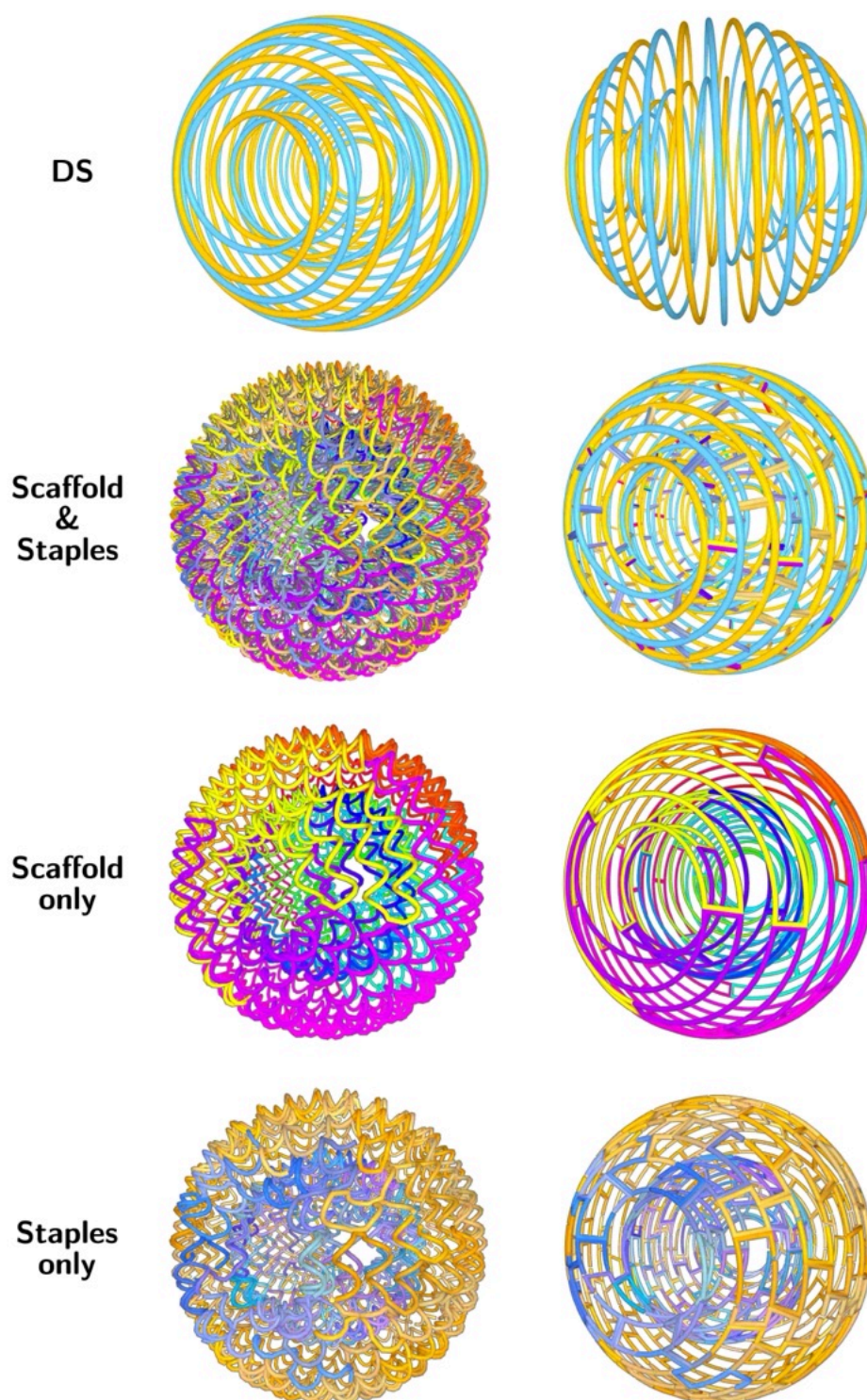


Figure S110: 3D views of the DS design: helices, scaffold, and staples. (Top row) Helix representation. (Other rows) From top to bottom: all strands, scaffold strand only, and staple strands only. Left and right columns show the full-strand and line representations, respectively.

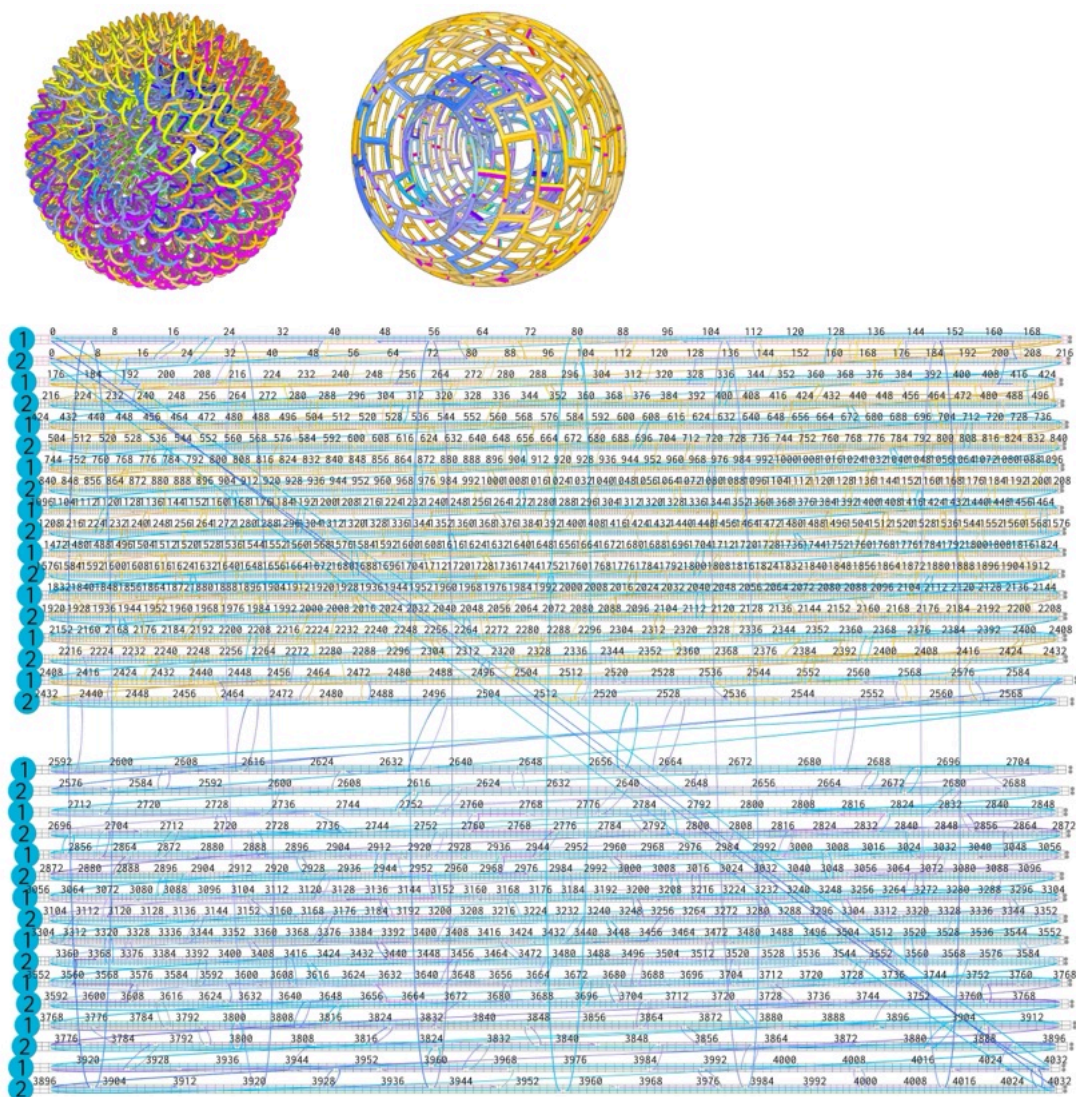


Figure S111: 2D view of the DS design. Scaffold and staples.



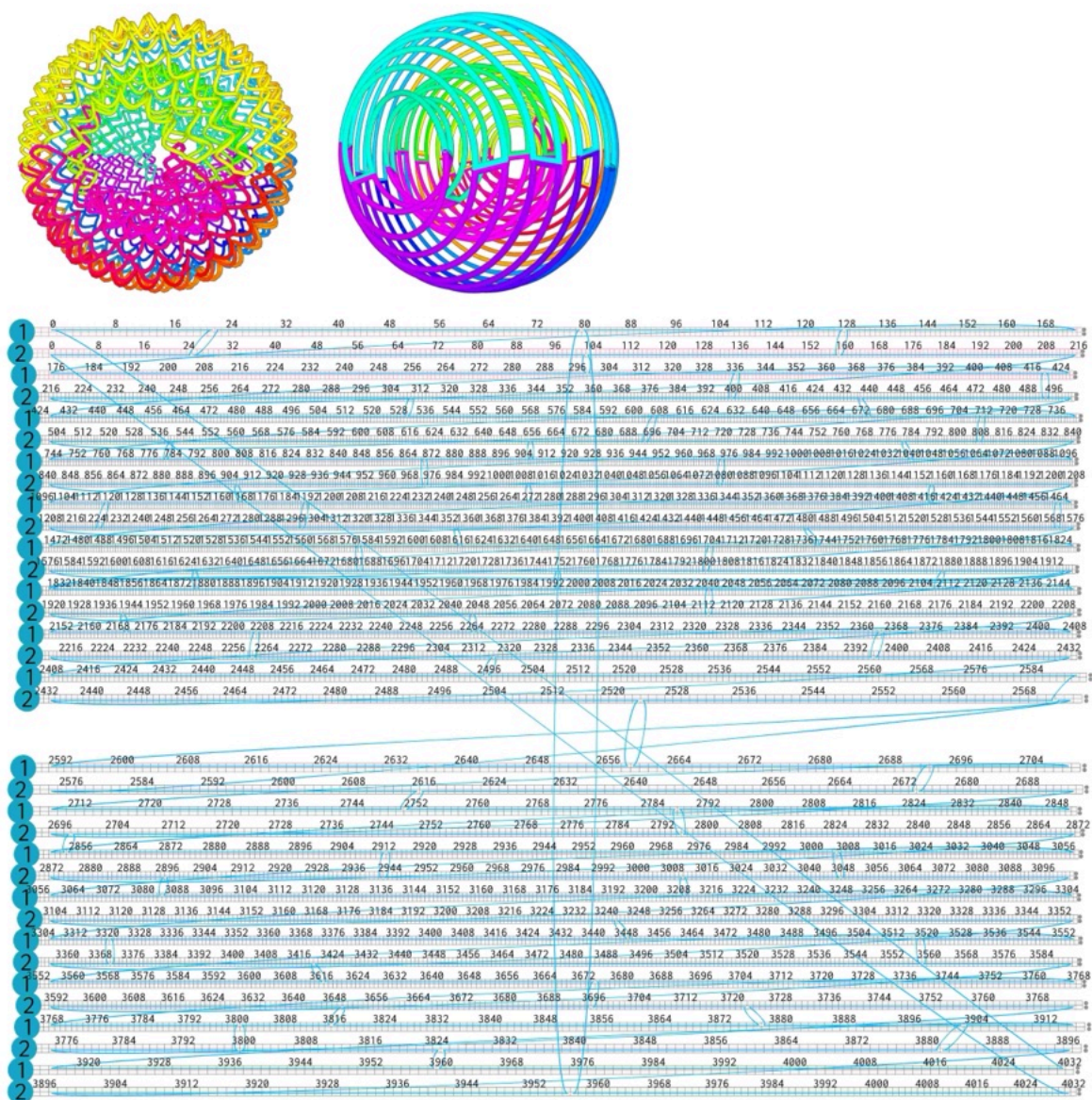


Figure S112: 2D view of the DS design. Scaffold only.

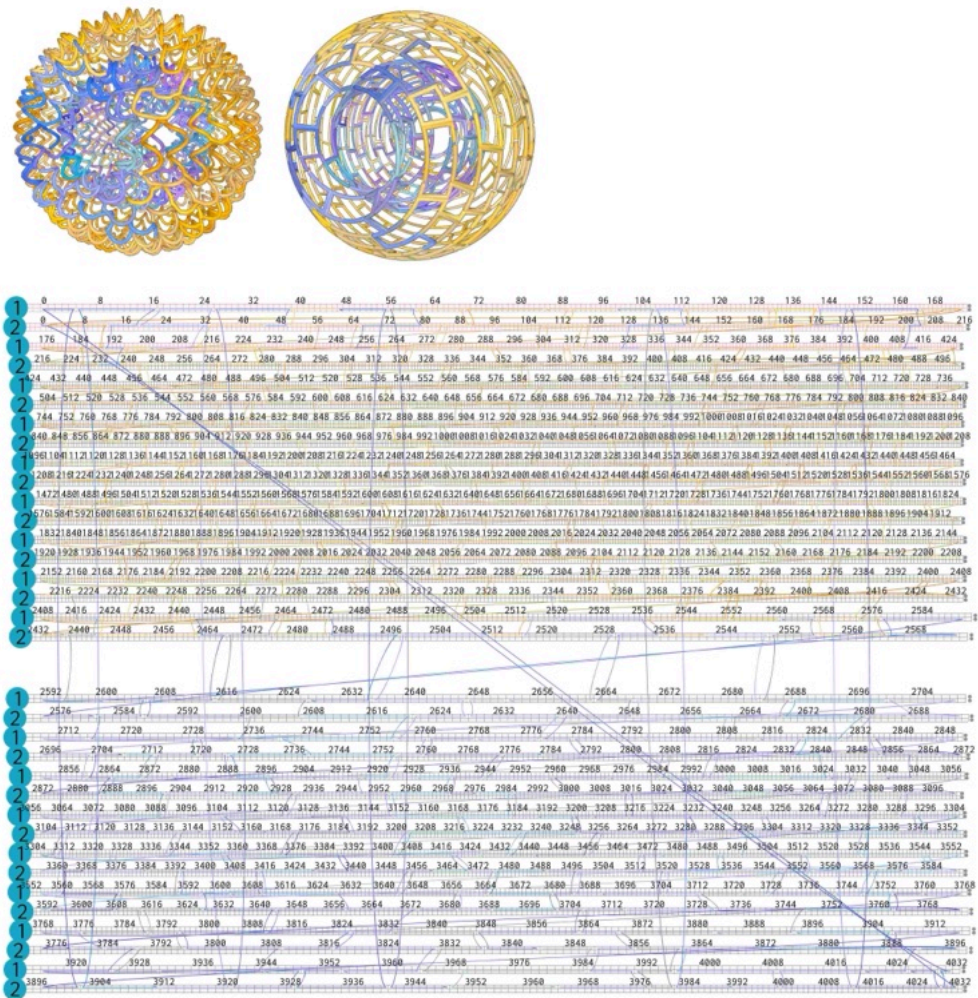


Figure S113: 2D view of the DS design. Staples only.

## S5.2 Double-sphere DS experimental validation

**Optimization of the folding conditions of the DS origami (fig. S114)** Three different folding ramps and three different magnesium concentrations were tested and analyzed by agarose gel electrophoresis to optimize the folding conditions. This analysis revealed a brighter monomeric band when the origami was folded over a long period of time (65°C for 15 min, 60°C to 20°C -1°C/2h, 10°C Hold, fig. S114) rather than over shorter folding ramps.

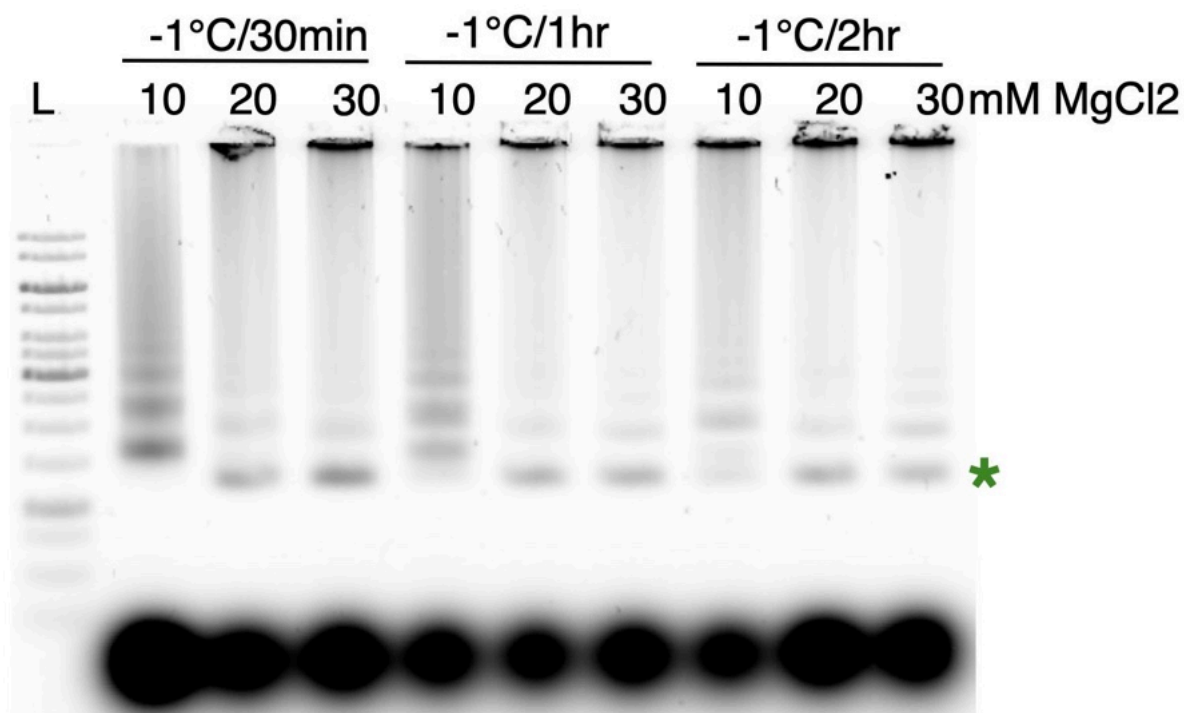


Figure S114: Gel analysis of the influence of  $MgCl_2$  concentration on folding quality of the DS. The foldings were analyzed by agarose gel electrophoresis (1%). L: 1kb DNA ladder. 65°C for 15 min, 60°C to 20°C -1°C/30min, 10°C Hold ; 65°C for 15 min, 60°C to 20°C -1°C/1h, 10°C Hold ; 65°C for 15 min, 60°C to 20°C -1°C/2h, 10°C Hold.



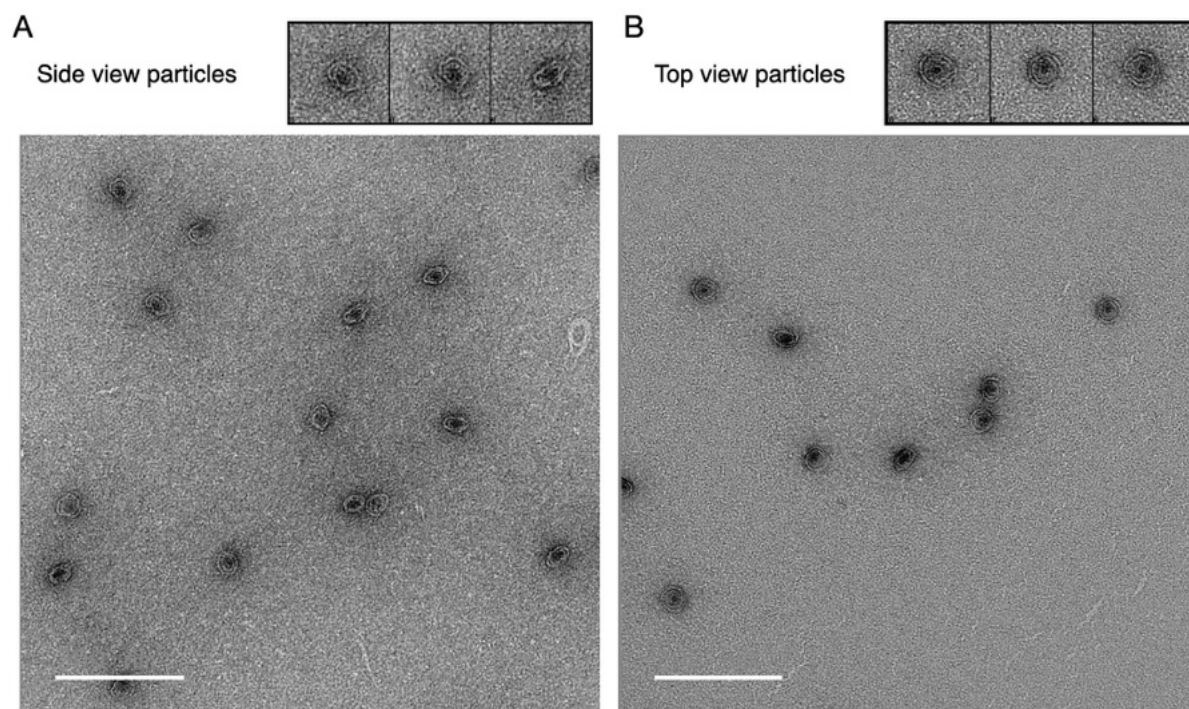


Figure S115: Transmission Electron Microscope (TEM) micrographs of typical particles of the DS. A: Selected side view particles. B: Selected Top view particles. Scale bar = 200 nm.

## S6 Vault-like origami, Figures S116 to S129

### S6.1 Vault-like origami design

Unlike the other origami structures, the vault design lacks the inner surface that would normally enclose an isolated internal compartment and mechanically reinforce the structure, making its successful assembly a particularly stringent test of the design methodology, particularly in the case of a single-layer architecture. Furthermore, unlike the other structures, whose cross sections are based on simple geometric primitives, the cross section of the vault is determined by its native shape. It is non-convex and contains multiple curvature inversions, further increasing the geometric complexity of the design.

The vault was designed in ENSnano by first defining a Bézier curve reproducing the cross section of the native vault structure extracted from the crystallographic model 2CV5.PDB. This curve was subsequently used as the cross-sectional profile in the “Surface of Revolution” tab (fig. S116). Because only the outer shell of the generated surface is retained in the final design, the target total size was set to 10,220 base pairs. Removal of the inner surface then yields a structure of the desired size of 8,064 base pairs. The complete design procedure is illustrated in fig. S117.

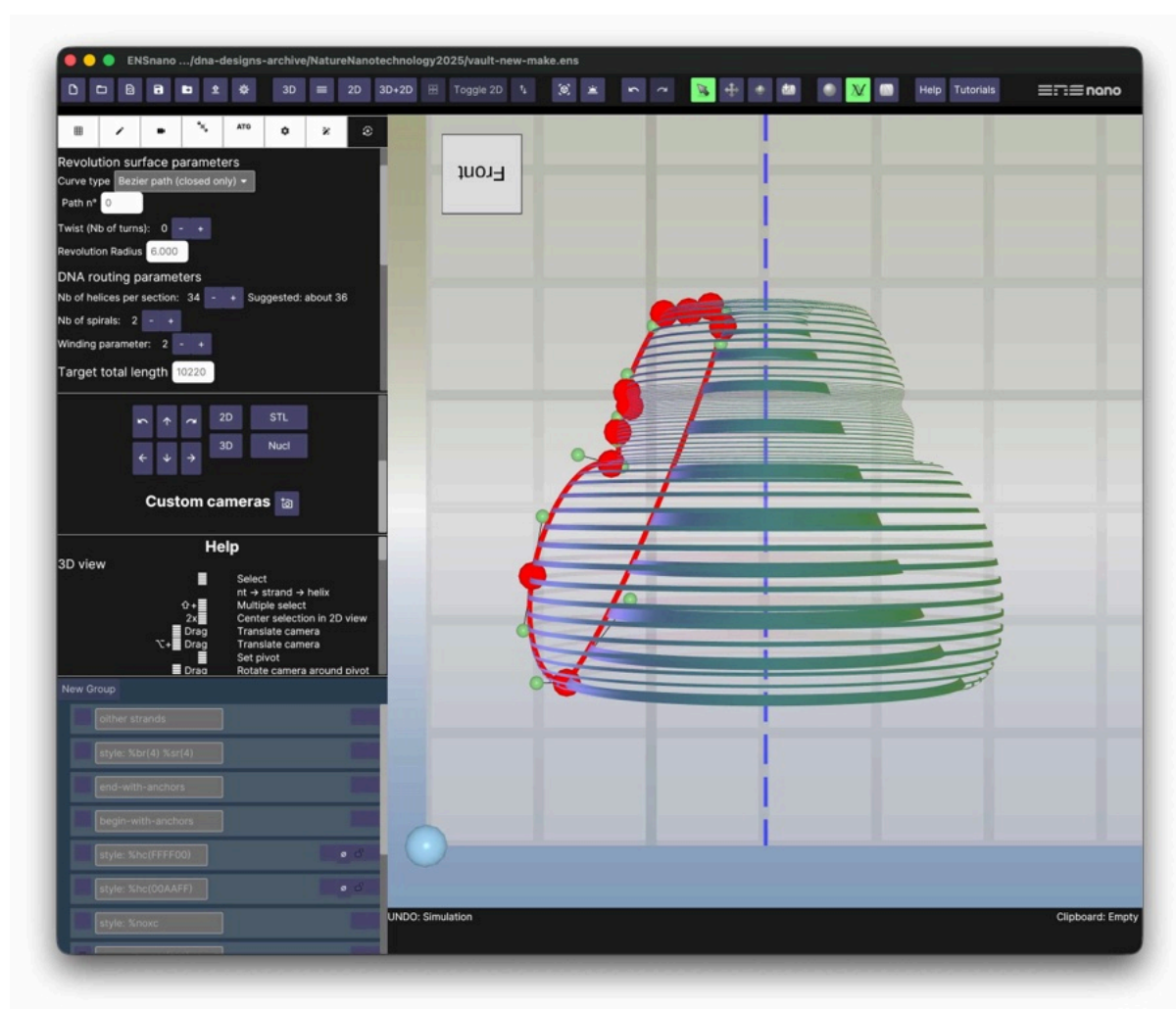
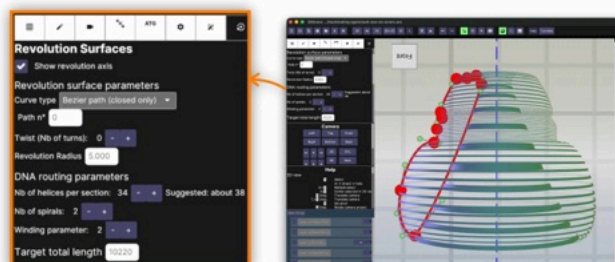


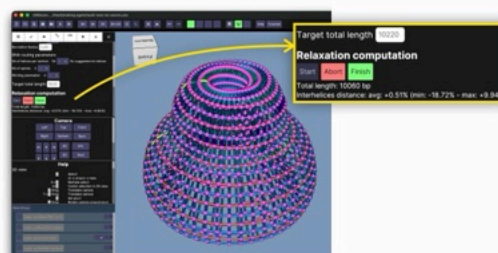
Figure S116: Surface-of-revolution, Bézier-profile, and helix-routing parameters of the vault design in ENSnano.



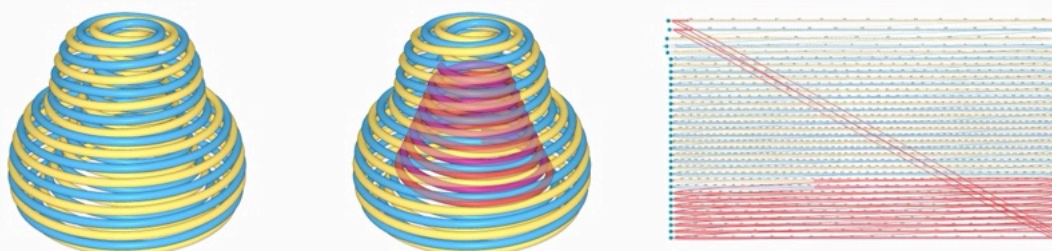
(1) Draw the cross-sectional Bézier curve and set the surface-of-revolution parameters



(2) Automatic optimization of the shape and helix routing to achieve a virtual total size of 10,220 bp (a few seconds)



(3) Identify and remove the portions of the two cyclic helices (5,110 bp each) corresponding to the inner surface (shown in red), measuring 1,030 bp and 1,126 bp in helices 1 and 2, respectively



(4) The resulting curved helices with a total size of 8,064 bp: 4,080 bp in helix 1 and 3,984 bp in helix 2



Figure S117: Step-by-step design of the vault origami with a target scaffold size of 8,064.

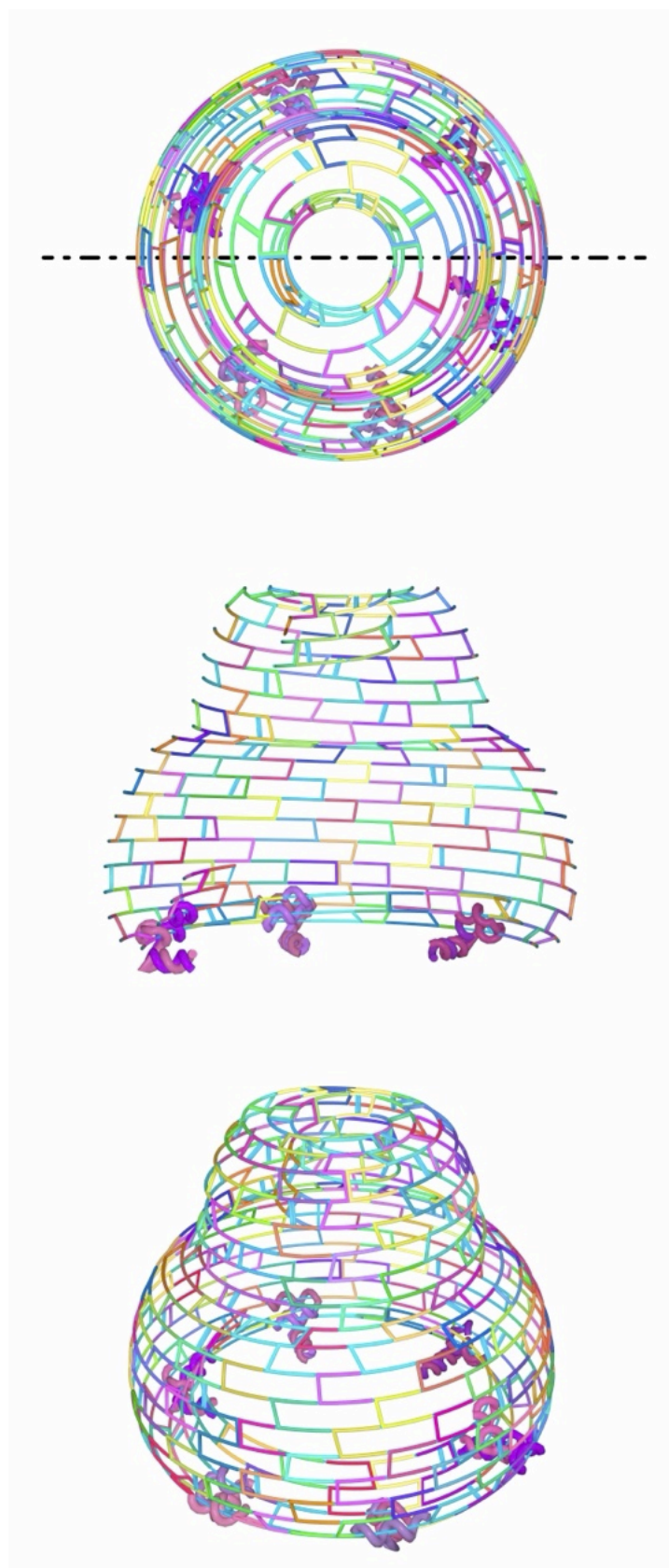


Figure S118: 3D view of the vault design (line representation). From top to bottom: top view, cross-sectional view (along the dashed line indicated in the top view), and three-quarter view. For clarity, the scaffold strand is rendered with a slightly thinner line, leaving only its crossover pattern visible. The six pairs of anchor staples used to promote homodimerization are shown in bold full-strand representation.

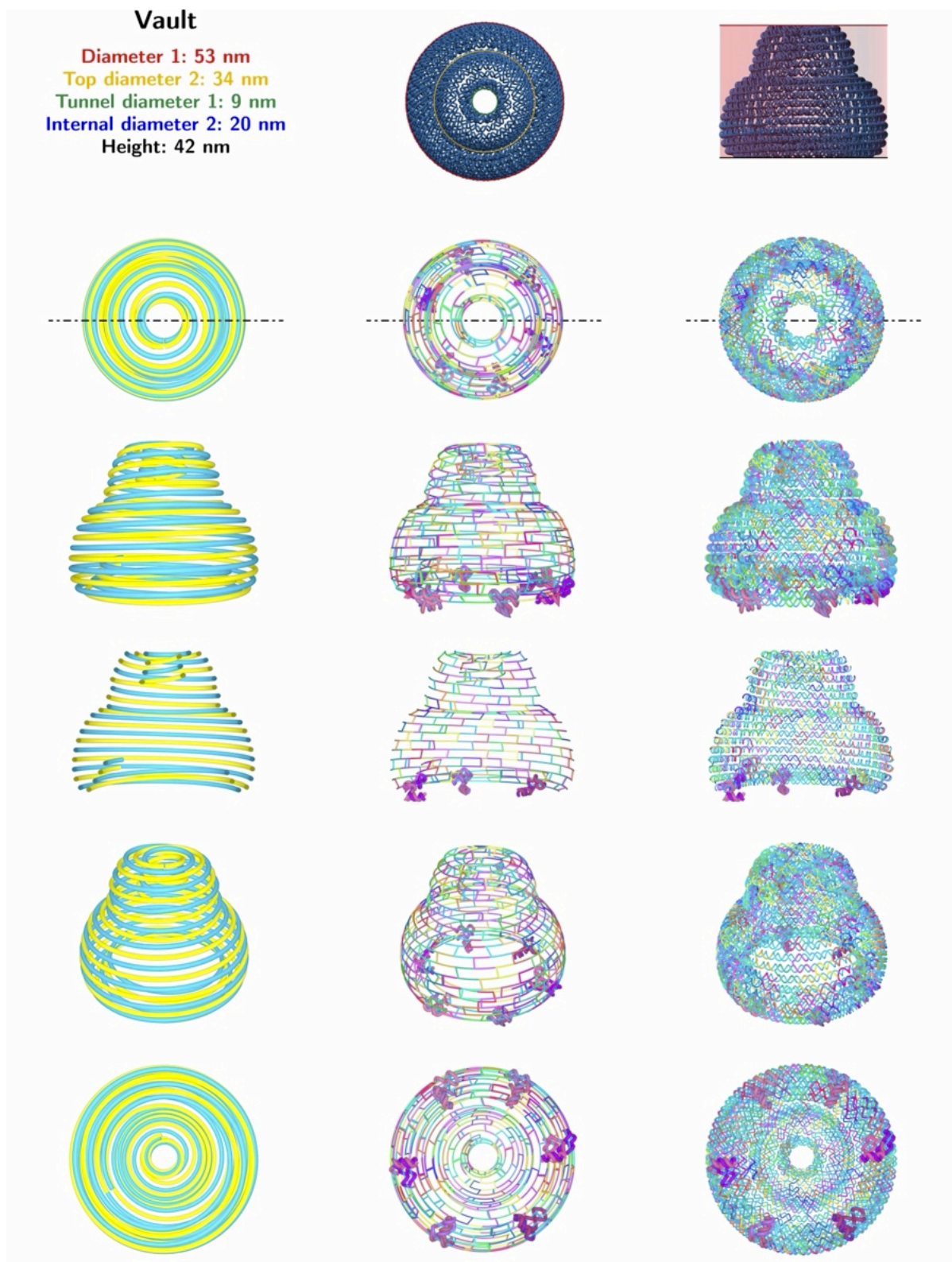


Figure S119: 3D views of the vault design (helix, line, and full-strand representations). (Top row) Dimensions of the vault design. The external diameter, upper diameter, and top-opening diameter are 53 nm (red), 34 nm (yellow), and 9 nm, respectively; the height is 42 nm. (Other rows) From top to bottom: top view, side view, cross-sectional view (along the dashed line indicated in the top view), three-quarter view, and bottom view. From left to right: helix, line, and full-strand representations. The six pairs of anchor staples used to promote homodimerization are shown in bold in the full-strand representation.



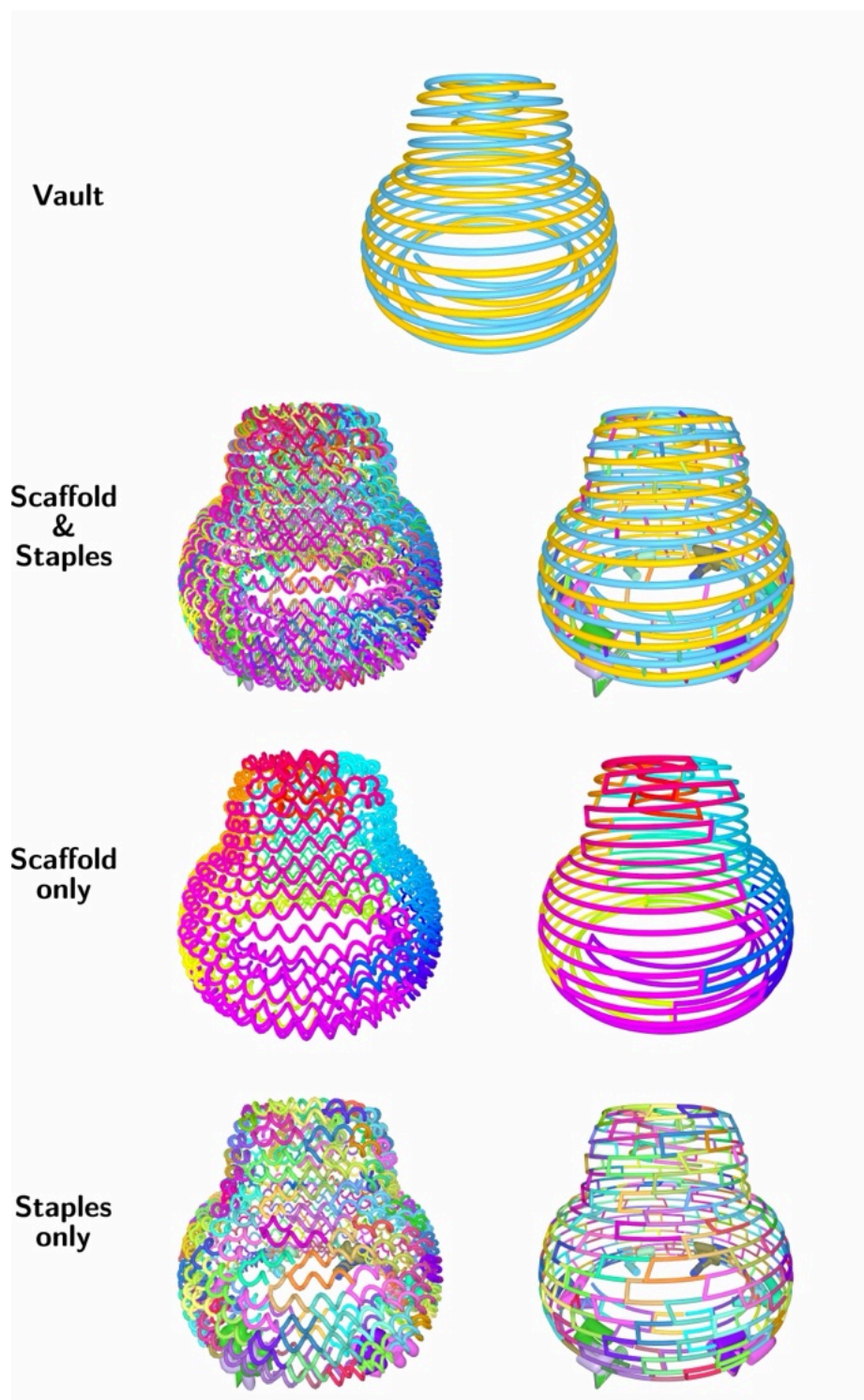
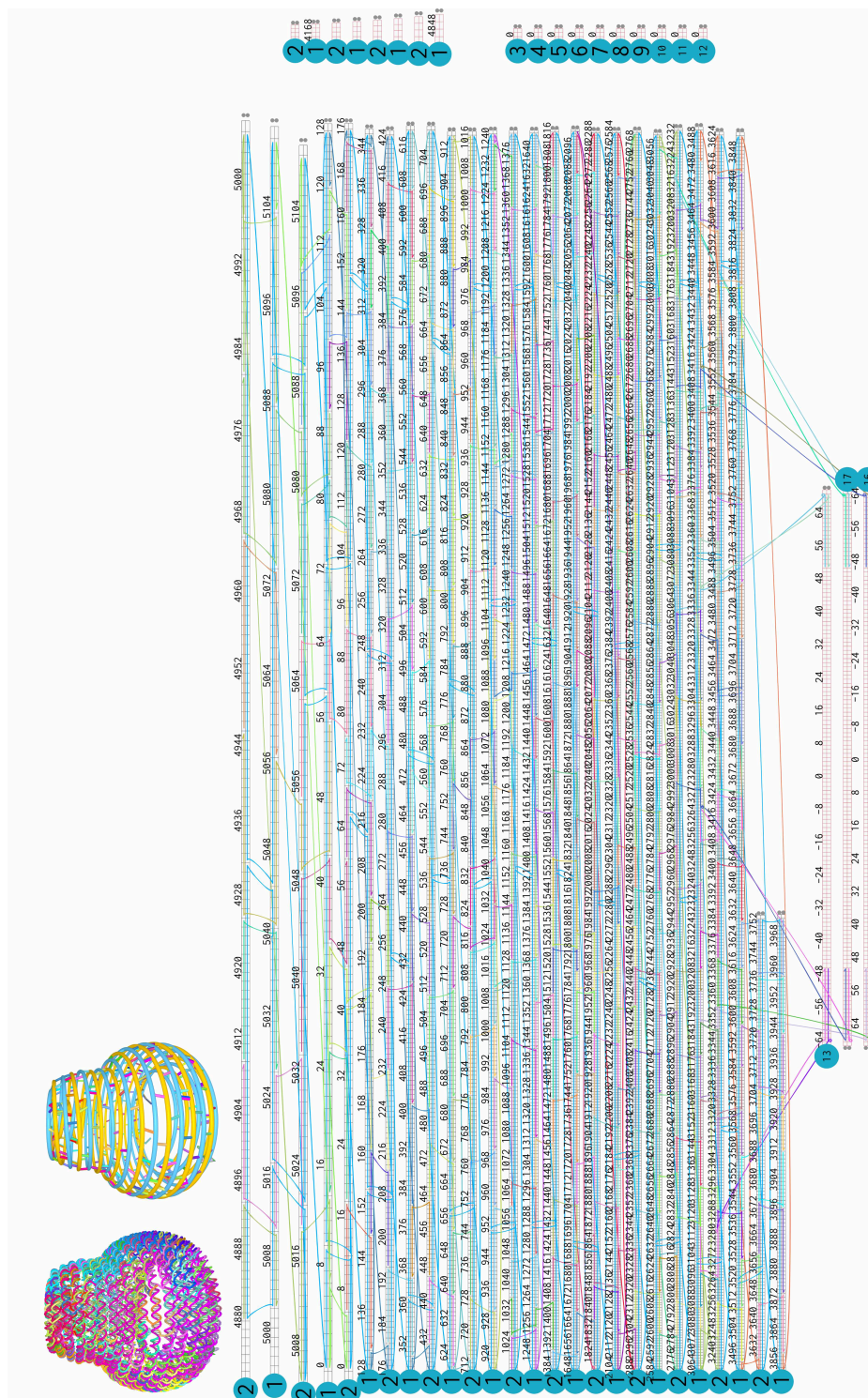


Figure S120: 3D views of the vault design: helices, scaffold, and staples. (Top row) Helix representation. (Other rows) From top to bottom: all strands, scaffold strand only, and staple strands only. Left and right columns show the full-strand and line representations, respectively. The six pairs of anchor staples used to promote homodimerization are shown in bold.





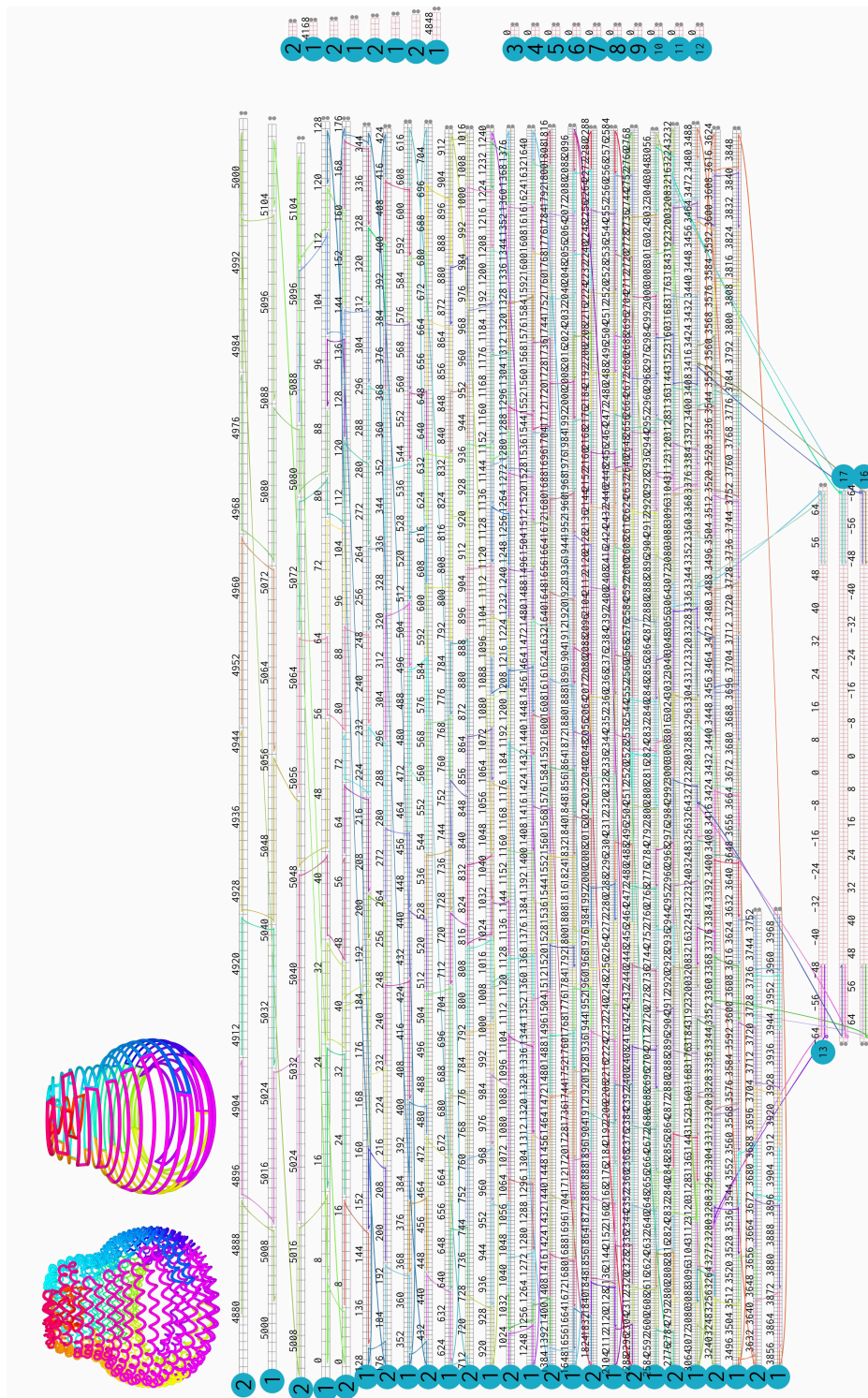


Figure S122: 2D view of the Vault-like design. Scaffold only.

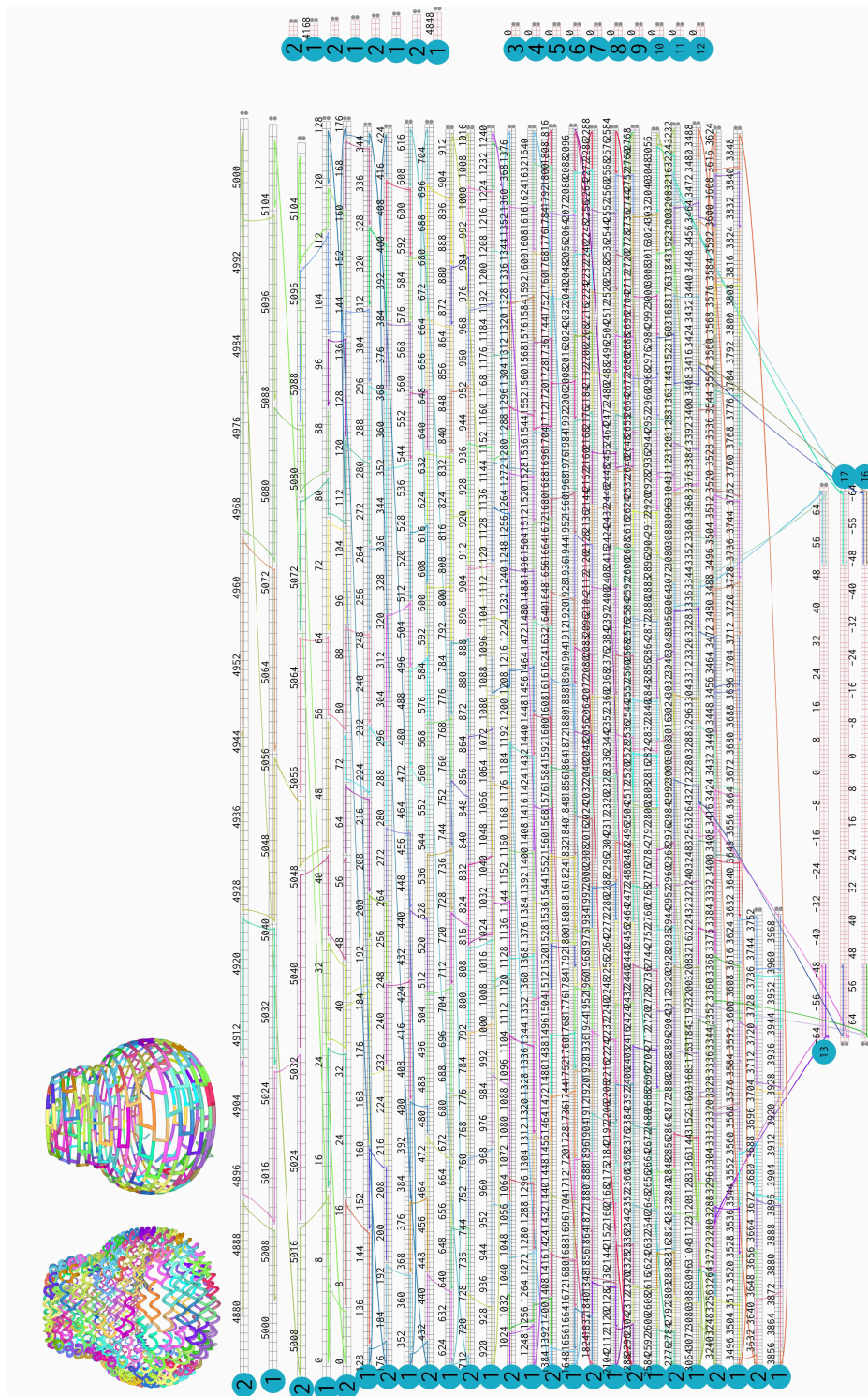


Figure S123: 2D view of the Vault-like design. Staples only.

## S6.2 Vault-like origami experimental validation

**Optimization of the folding conditions of the Vault origami (figs. S124 to S129)** Three different folding ramps (over 3.5 hours, 33 hours and 42 hours) were first tested and analyzed by agarose gel electrophoresis, with six different magnesium concentrations (10 to 20 mM) and a scaffold concentration of 20 nM with staples oligonucleotides in a 10-fold excess (fig. S124). The gel showed that the yield was better with higher magnesium concentrations and longer folding ramps.

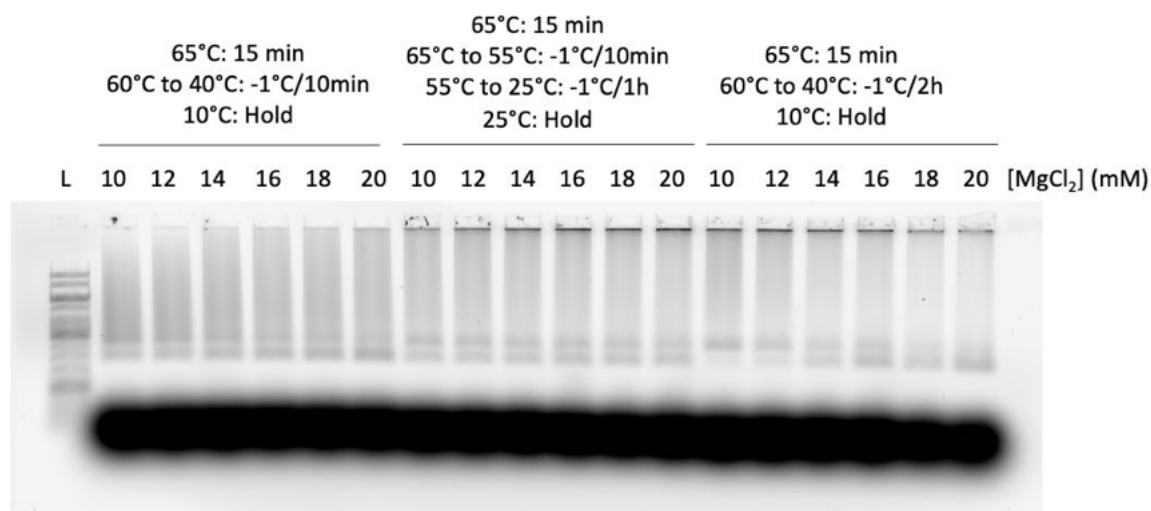


Figure S124: Gel analysis of the influence of  $\text{MgCl}_2$  concentration and folding ramp on folding quality of the Vault. The foldings were analyzed by agarose gel electrophoresis (1%). L: 1kb DNA ladder.

1442 We then folded the vault with 20 mM of magnesium over a 63-hour time period and compared it to the  
 1443 foldings of the vault over a 42-hour and over a 3.5-hour time period thanks to agarose gel electrophoresis  
 1444 (fig. S125). For all the conditions, we observed a monomeric lower band, with a better yield with the  
 1445 longest folding ramp (63 hours).

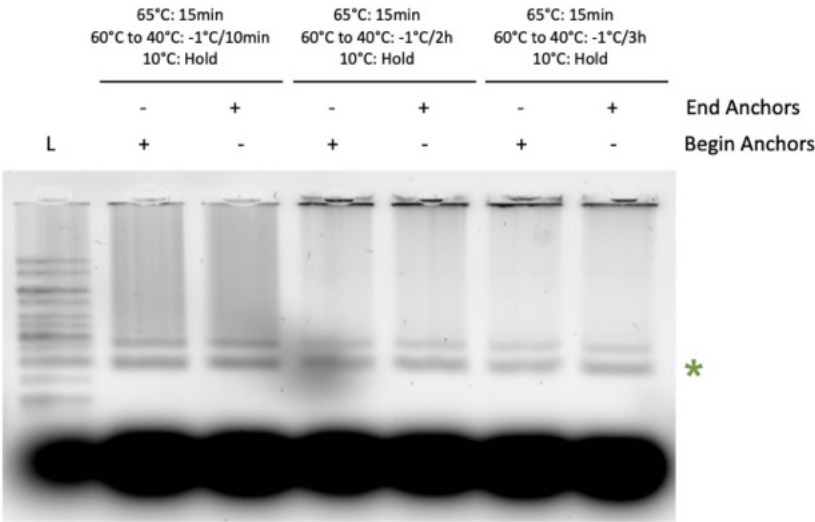


Figure S125: Gel analysis of the influence of different folding ramps on the folding quality of the Vault. For each folding ramp, the two versions of the Vault monomer (with 5'-anchors: "Begin Anchors" and with 3'-anchors: "End Anchors") designed to enable the dimerization were folded. The foldings were analyzed by agarose gel electrophoresis (1%). L: 1kb DNA ladder.



We analyzed the purified monomers of the Vault folded with these three conditions with negative staining electron-microscopy. The micrographs, galleries and 2D-class averaging displayed anticipated shapes of the Vault-like origami even though the 3.5-hour and 42-hour long folding ramps showed monomers which looked more flattened than the ones observed with the 63-hour long folding ramp (Figure 4, fig. S126).

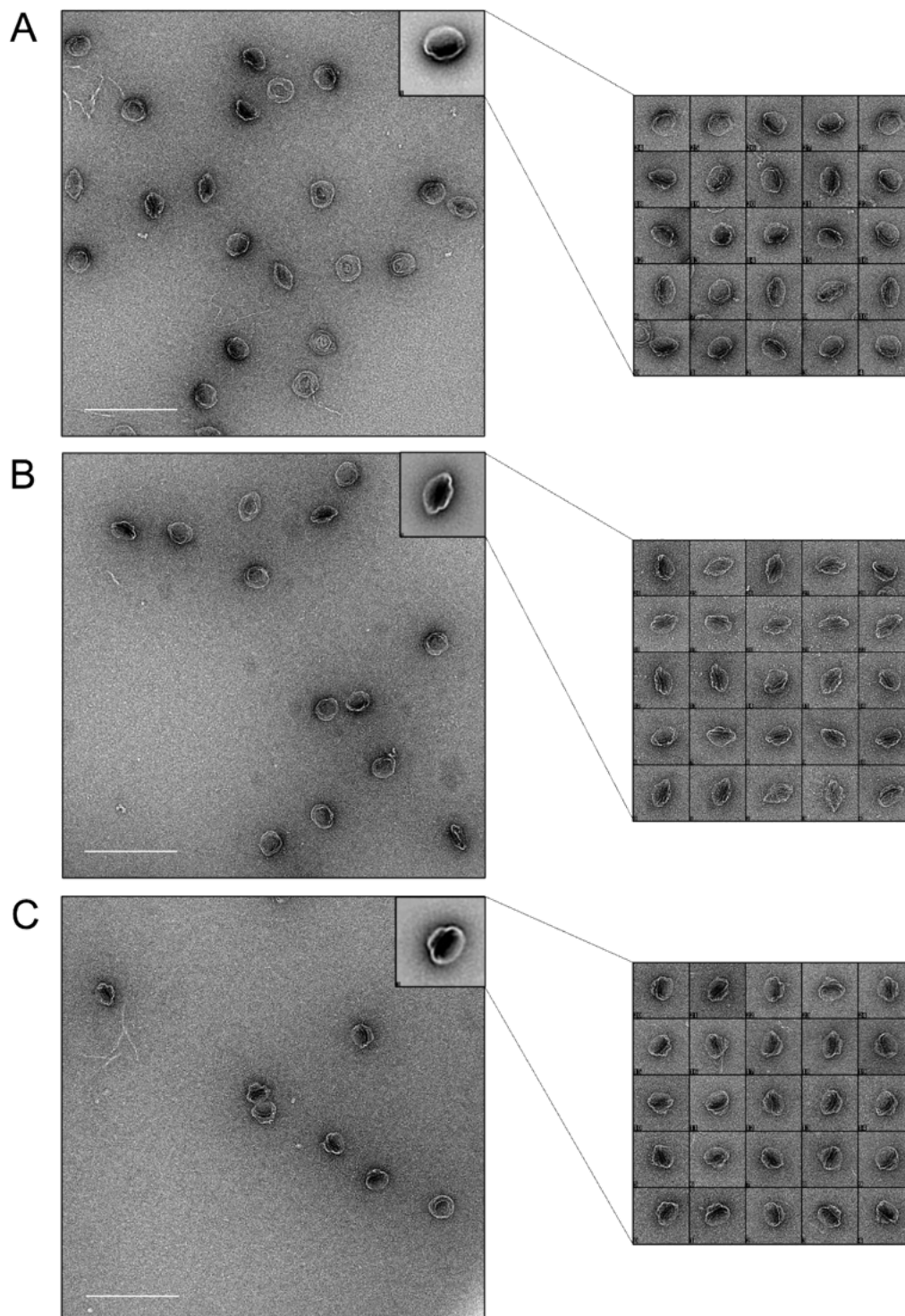


Figure S126: ns-TEM analysis of purified Vault monomers folded with different folding ramps. For each condition, one TEM micrograph, a 2D-class averaging and a gallery of typical particles observed on micrographs are depicted. A: 65°C for 15 min, 60°C to 40°C -1°C/10min, 10°C Hold; B: 65°C for 15 min, 60°C to 40°C -1°C/2h, 10°C Hold; C: 65°C for 15 min, 60°C to 40°C -1°C/3h, 10°C Hold. Scale bar = 200 nm. Box size = 112 nm x 112 nm.

1451 After optimization of the dimerization conditions (fig. S127), we incubated the Vault monomers  
 1452 obtained with each of the three previously mentioned folding ramps with 100 mM magnesium at 37°C  
 1453 over a 15-hour time period. We then analyzed the dimers formation by agarose-gel electrophoresis  
 1454 (Figure 4, fig. S128) and ns-TEM (Figure 4, fig. S129).

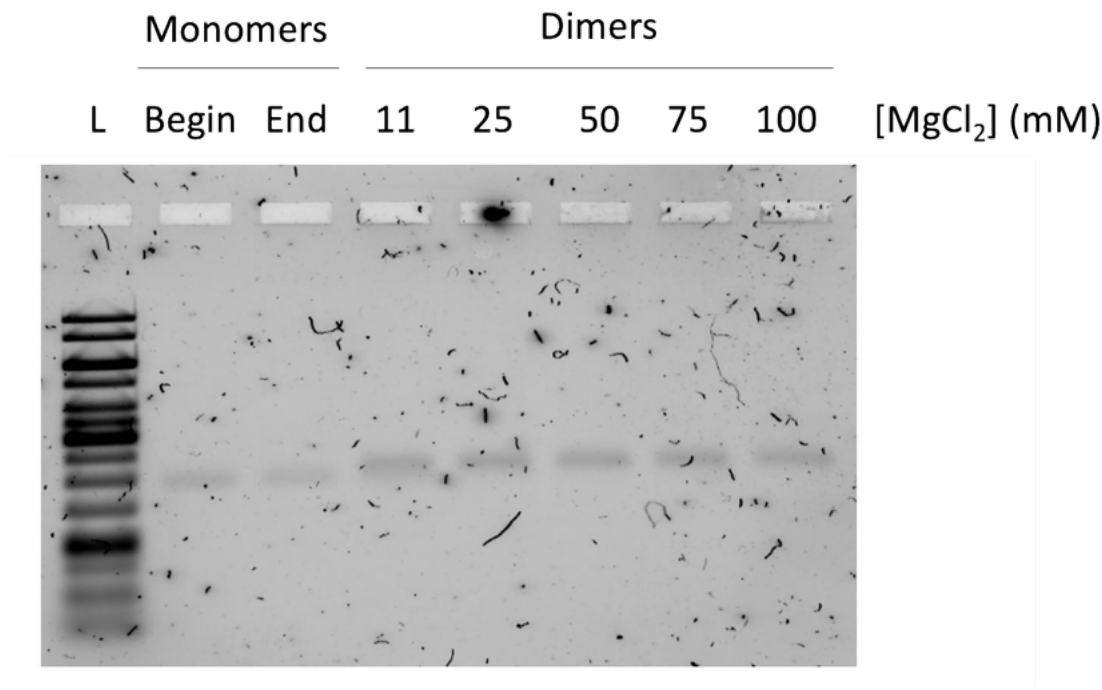


Figure S127: Gel analysis of the influence of different MgCl<sub>2</sub> concentrations on the dimerization of the vault-origami. The dimerization quality was analyzed by agarose gel electrophoresis (1%). The monomers “Begin” and “End” correspond to the vault monomers with 5'-anchors and 3'-anchors respectively, after a 42-hour folding and gel purification. L: 1kb DNA ladder. The monomers “Begin” and “End” were incubated with different MgCl<sub>2</sub> concentrations over a 15-hour time period at 37°C to enable dimerization. We observe retarded bands for the dimers compared to the monomers, suggesting some oligomerization (possibly dimerization). At 100 mM MgCl<sub>2</sub>, we observe a slightly retarded band compared to the other ones, suggesting a better dimerization of the monomers.

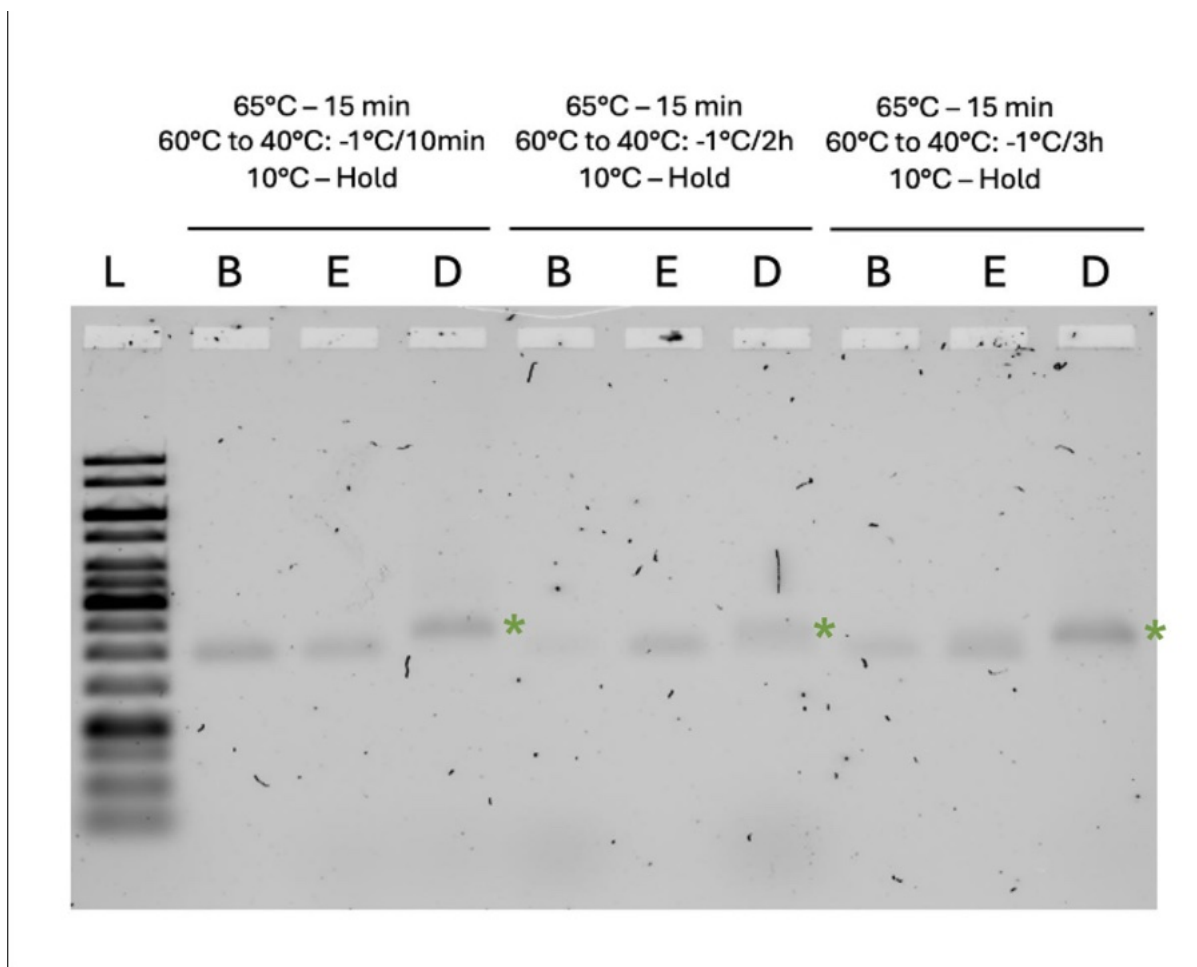


Figure S128: Gel analysis of the influence of the folding ramp of the vault monomers on their dimerization. The dimerization quality was analyzed by agarose gel electrophoresis (1%). B: purified vault monomers with 5'-anchors; E: purified vault monomers with 3'-anchors; D: obtained dimer by incubating B and E with 100 mM MgCl<sub>2</sub> over a 15-hour time period at 37°C; L: 1kb DNA ladder. We observe that for all the folding ramps used to fold the monomers, retarded bands were observed after incubation of the monomers together (D), suggesting some oligomerization (possibly dimerization).

The observed dimers formed from monomers folded over a 63-hour time period appeared to be longer than the ones formed from monomers folded with the two other folding ramps (fig. S129).

Hence, we concluded that the optimal folding ramp for the folding of the vault origami was the 63-hour long folding ramp.

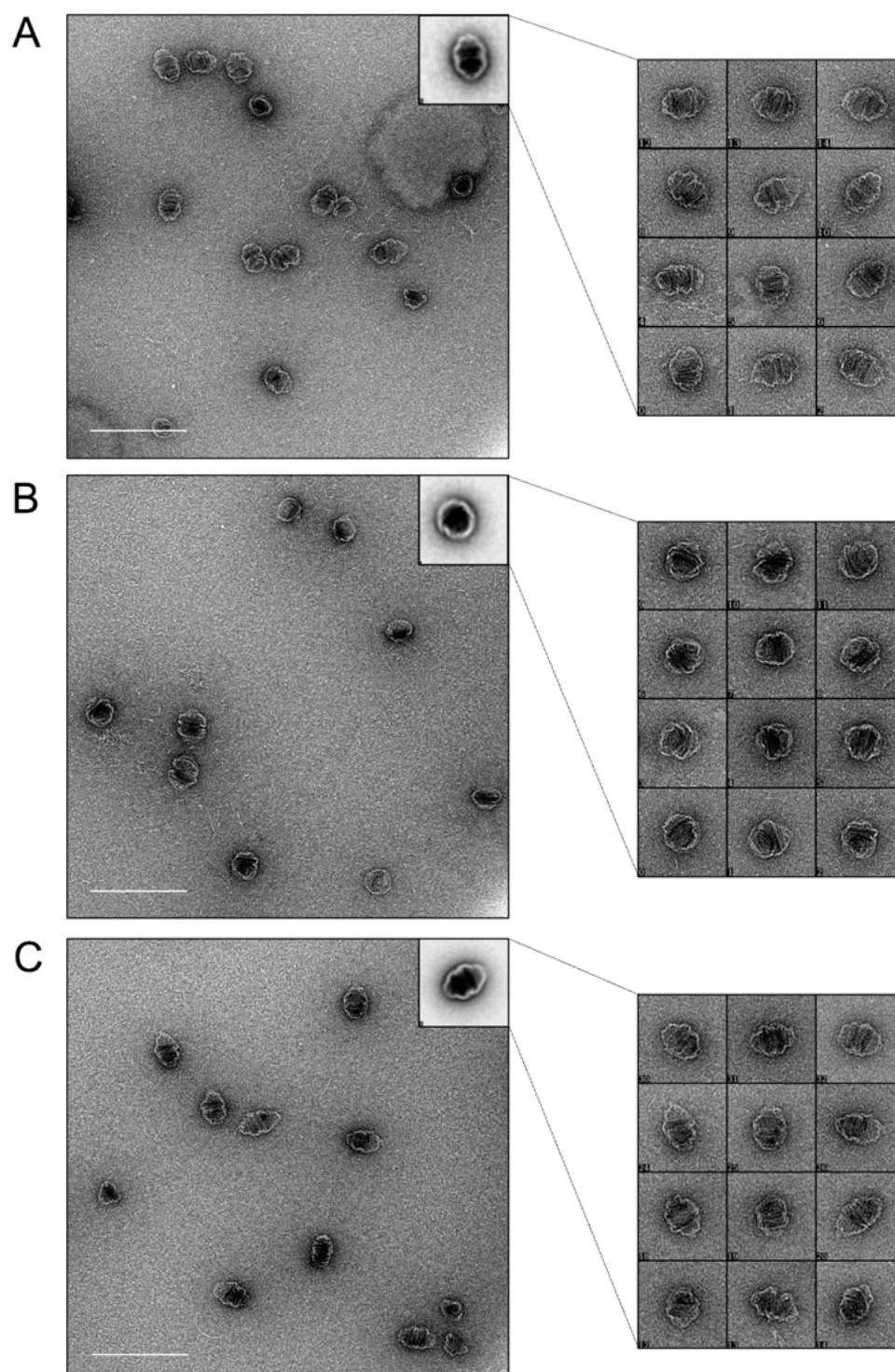


Figure S129: ns-TEM analysis of purified Vault dimers obtained from vault monomers folded with different folding ramps. For each condition, one TEM micrograph, a 2D-class averaging and a gallery of typical particles observed on micrographs are depicted. A: 65°C for 15 min, 60°C to 40°C -1°C/10min, 10°C Hold; B: 65°C for 15 min, 60°C to 40°C -1°C/2h, 10°C Hold; C: 65°C for 15 min, 60°C to 40°C -1°C/3h, 10°C Hold. Scale bar = 200 nm. Box size = 132 nm x 132 nm.



# S7 Summary of all the designs and design parameters, Figures S130 and S131



Figure S130: All the designs and their expected sizes

T1:

Revolution surface parameters

Curve type Ellipse

Semi major axis 10.8

Semi minor axis 5.4

Twist (Nb of 1/2-turns): 1

Revolution Radius 19.700

DNA routing parameters

Nb of helices per section: 20 Suggested: about 16

Nb of spirals: 2

Winding parameter: -4

Target total length 7560

T2:

Revolution surface parameters

Curve type Ellipse

Semi major axis 10

Semi minor axis 5

Twist (Nb of 1/2-turns): 2

Revolution Radius 21.100

DNA routing parameters

Nb of helices per section: 18 Suggested: about 16

Nb of spirals: 2

Winding parameter: -4

Target total length 7560

T3:

Revolution surface parameters

Curve type Ellipse

Semi major axis 9.1

Semi minor axis 4.55

Twist (Nb of 1/2-turns): 3

Revolution Radius 22.200

DNA routing parameters

Nb of helices per section: 16 Suggested: about 14

Nb of spirals: 2

Winding parameter: -6

Target total length 7560

T4:

Revolution surface parameters

Curve type Ellipse

Semi major axis 9.6

Semi minor axis 4.8

Twist (Nb of 1/2-turns): 4

Revolution Radius 22.200

DNA routing parameters

Nb of helices per section: 16 Suggested: about 16

Nb of spirals: 2

Winding parameter: -10

Target total length 7560

T5:

Revolution surface parameters

Curve type Ellipse

Semi major axis 9

Semi minor axis 4.5

Twist (Nb of 1/2-turns): 5

Revolution Radius 24.450

DNA routing parameters

Nb of helices per section: 14 Suggested: about 14

Nb of spirals: 2

Winding parameter: -11

Target total length 7560

T6:

Revolution surface parameters

Curve type Ellipse

Semi major axis 8.7

Semi minor axis 4.35

Twist (Nb of 1/2-turns): 6

Revolution Radius 24.000

DNA routing parameters

Nb of helices per section: 14 Suggested: about 14

Nb of spirals: 2

Winding parameter: -12

Target total length 7560

DS:

Revolution surface parameters

Curve type Two spheres

Radius extern 25.000

Radius intern 17.000

Radius tube 7.600

Smooth cell 0.040

Twist (Nb of turns): 0

Revolution Radius 15.000

DNA routing parameters

Nb of helices per section: 34 Suggested: about 28

Nb of spirals: 2

Winding parameter: 2

Target total length 8064

vault:

Revolution surface parameters

Curve type Bezier path (closed only)

Path n° 0

Twist (Nb of turns): 0

Revolution Radius 6.000

DNA routing parameters

Nb of helices per section: 34 Suggested: about 36

Nb of spirals: 2

Winding parameter: 2

Target total length 10220

Figure S131: Summary of the geometric and routing parameters used to design the origami structures based on (possibly twisted) surfaces of revolution.

## S8 Distribution of staple strand crossover spacing, Figures S132 to S146

Figures S132, S134, S136, S138, S140, S142, S144 and S146 show the distribution of staple crossover spacing for the designs  $\aleph$ , T2, T3, T4, T5, T6, DS, and a classic flat square design. The colors of the bars indicate the average curvature radius (main color scale: 4 nm [red] to 40 nm [blue]) and average of the absolute value of torsion (inset color scale: 0/nm [blue] to 0.2/nm [red]) at the crossover locations. As opposed to the classic flat design, where spacing are sharply centered at 16 and 31 nucleotides (fig. S146), the crossover spacing in our curved and twisted designs are broadly distributed, forming three main peaks centered around 6–8, 15, and 25 nucleotides. The spike at 1 nucleotide corresponds to double crossovers.

Note that the different curvature radii and torsion values are similarly represented within the two peaks centered at 15 and 25 nucleotides across all designs. However, the peak centered at 4–6 nucleotides appears to include crossovers located at regions of smaller curvature radius (e.g., fig. S144). This observation may serve as a design guideline: regions with smaller curvature radii may require more closely spaced crossovers.

Finally, these analyses suggest that accommodating curvature and torsion requires a broader distribution of crossover spacings, an outcome enabled by our spiral design paradigm. Moreover, no systematic relationship is observed between crossover spacing and either the local curvature radius or torsion.

As shown in Figures S133, S135, S137, S139, S141, S143 and S145, both Pearson and Spearman correlation coefficients between crossover spacing, curvature radius, and torsion remain close to zero across all structures.

These results indicate that local crossover placement cannot be reliably inferred from simple geometric descriptors using a rule-based strategy. Instead, they highlight the importance of our three-dimensional nucleotide-positioning approach for accurate crossover design in curved DNA nanostructures.

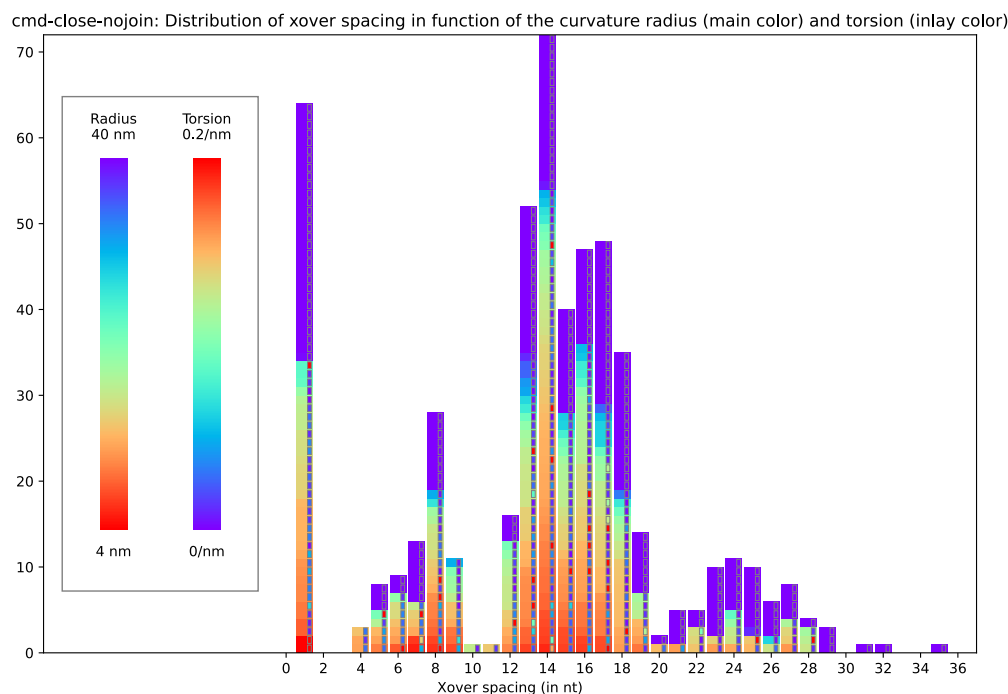


Figure S132: Distribution of staple strand crossover spacing correlated (in colors) with local curvature radius and torsion in the  $\aleph$ -origami.

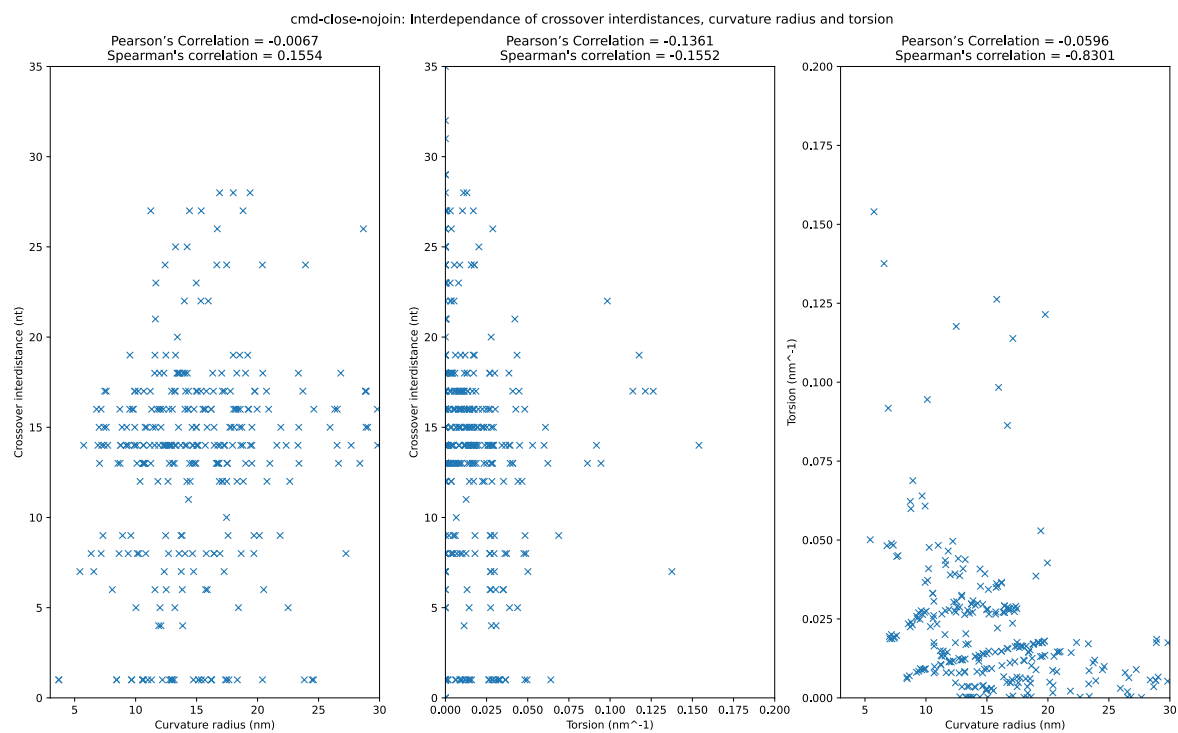


Figure S133: Evaluation of the correlations between crossover spacing, local curvature radius, and torsion in the ⌘-origami.

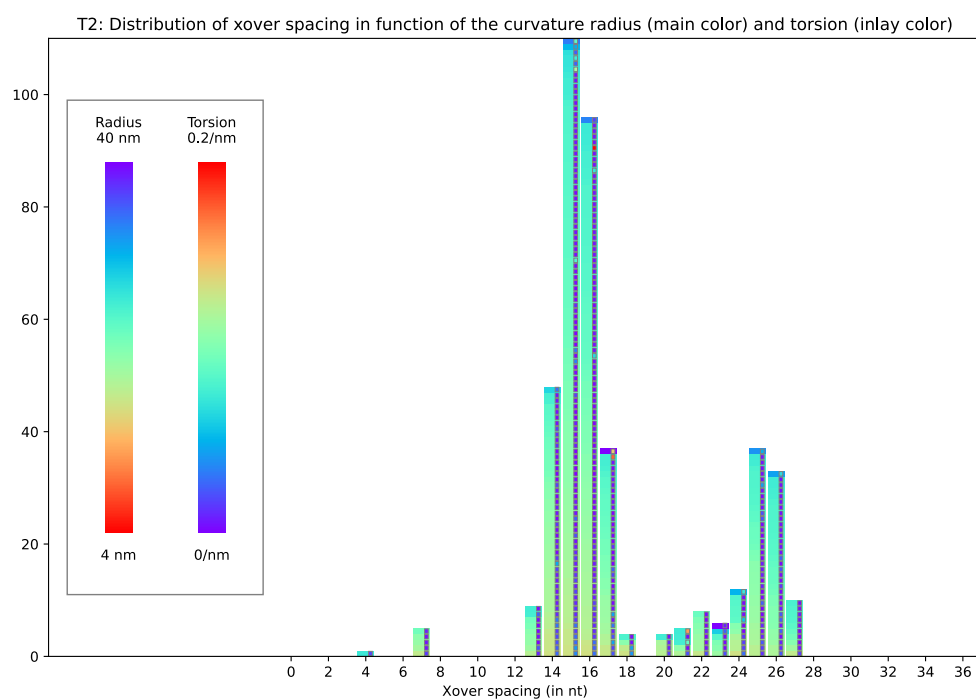


Figure S134: Distribution of staple strand crossover spacing correlated (in colors) with local curvature radius and torsion in the T2-origami.



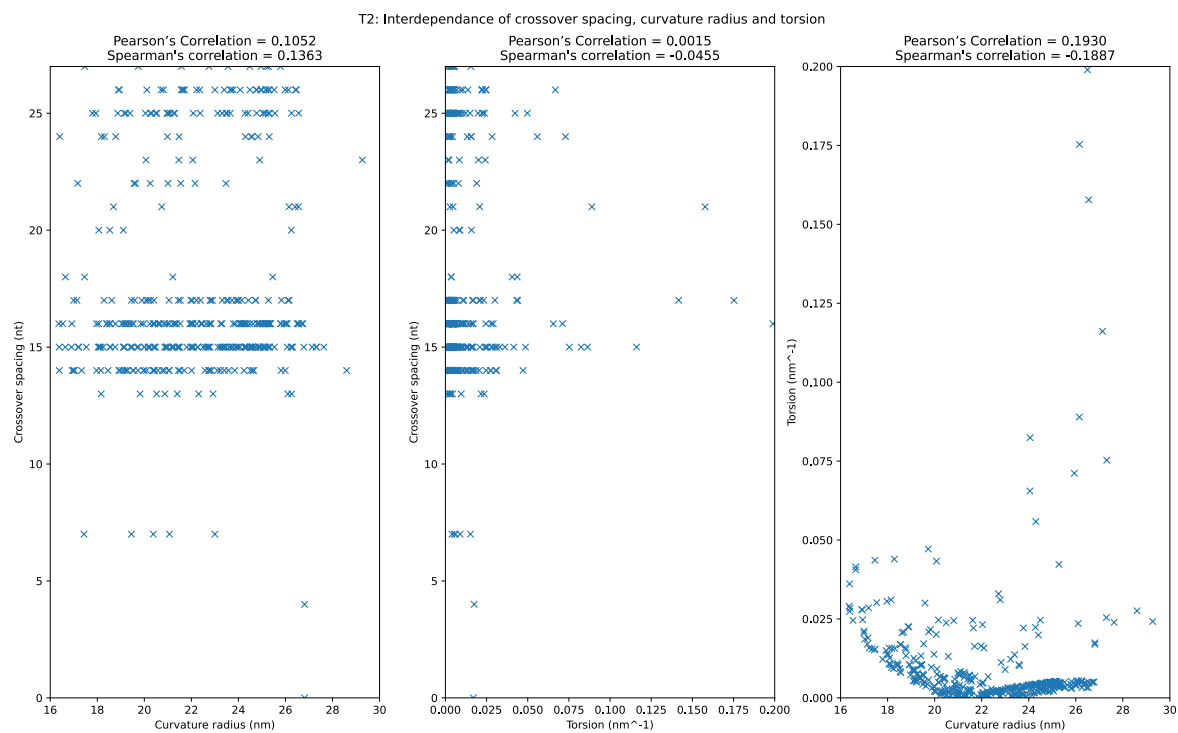


Figure S135: Evaluation of the correlations between crossover spacing, local curvature radius, and torsion in the T2-origami.

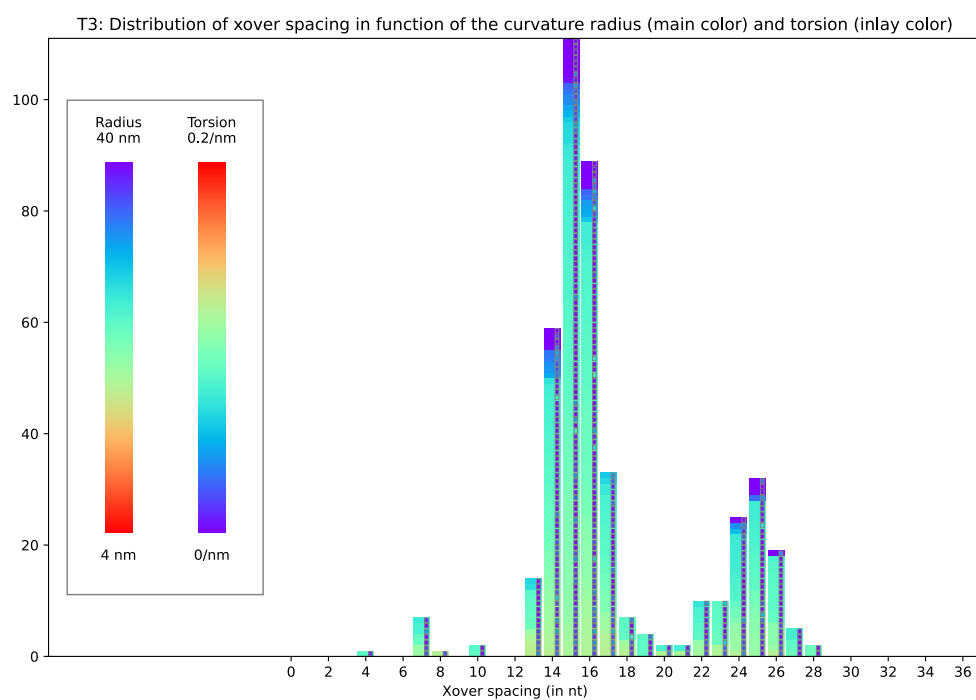


Figure S136: Distribution of staple strand crossover spacing correlated (in colors) with local curvature radius and torsion in the T3-origami.

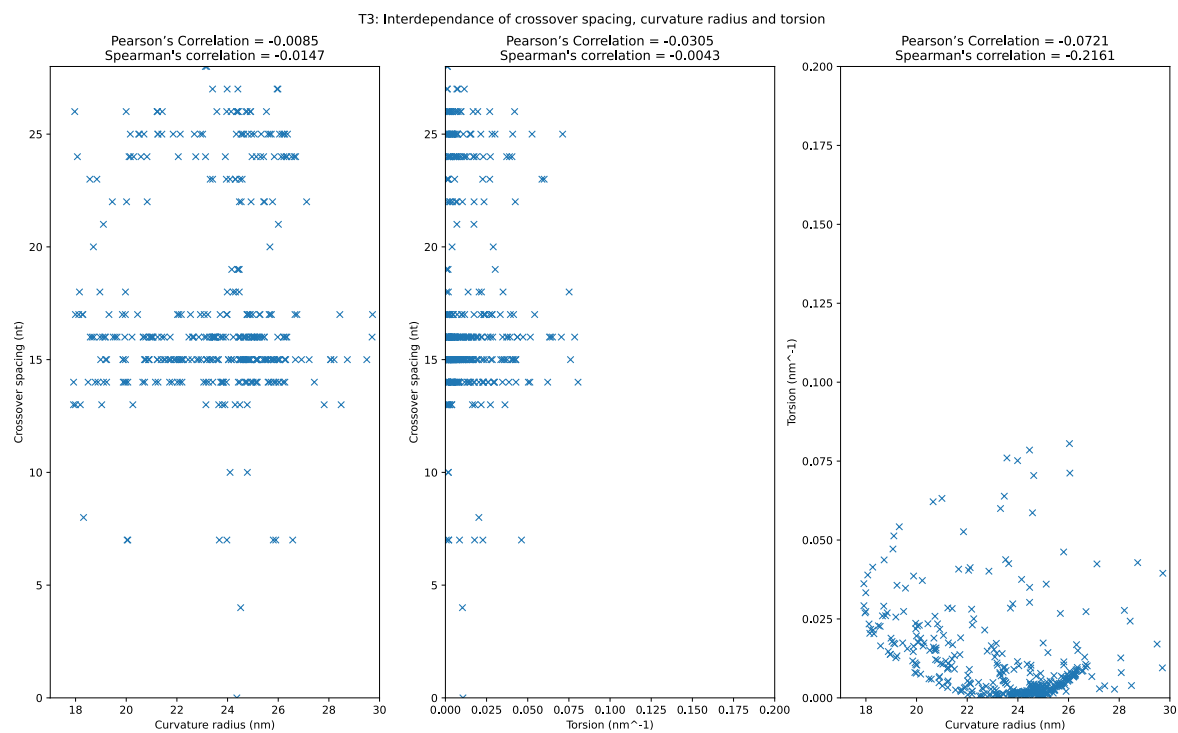


Figure S137: Evaluation of the correlations between crossover spacing, local curvature radius, and torsion in the T3-origami.

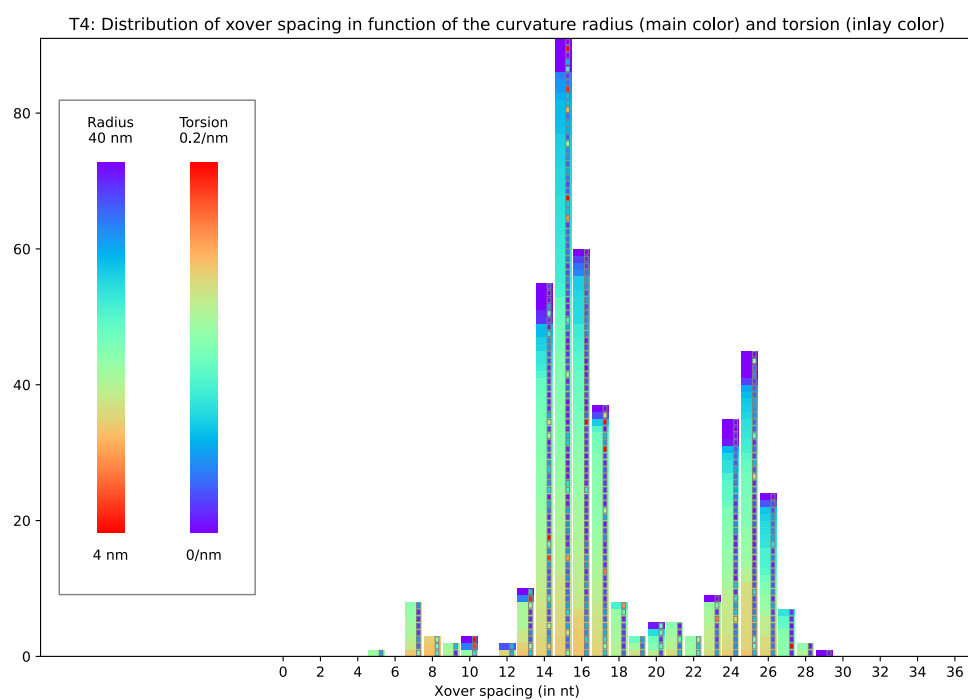


Figure S138: Distribution of staple strand crossover spacing in the T4-origami.

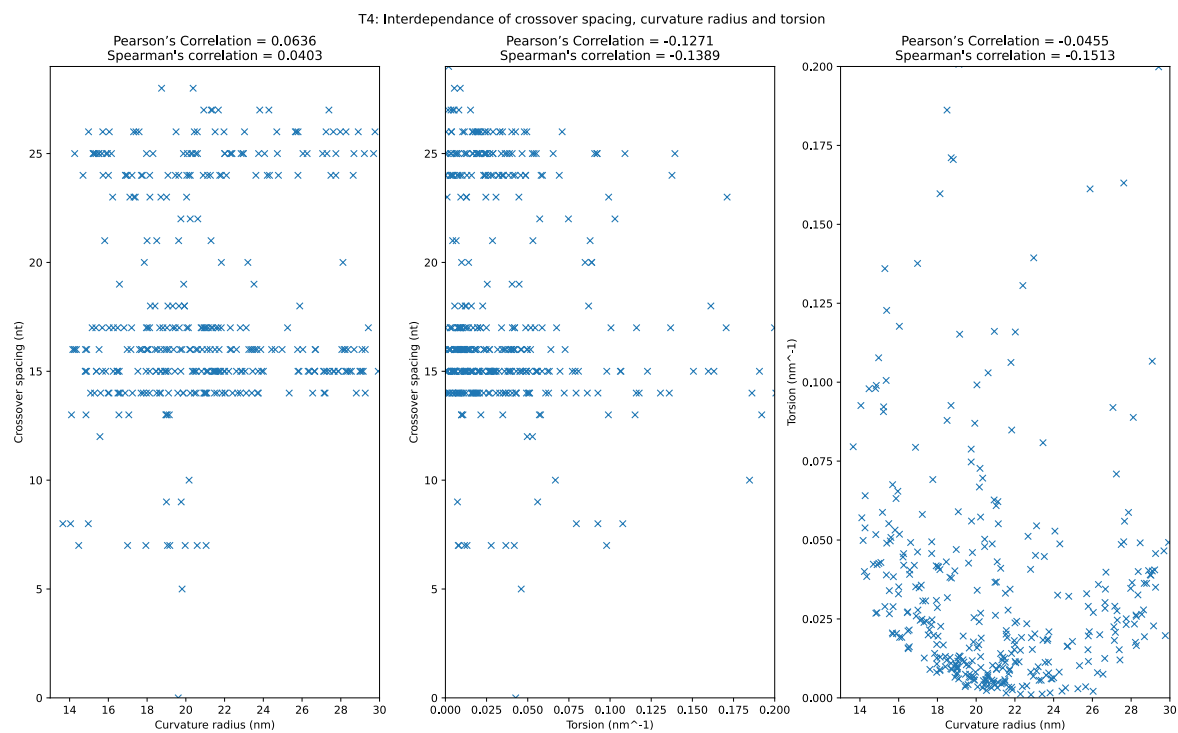


Figure S139: Evaluation of the correlations between crossover spacing, local curvature radius, and torsion in the T4-origami.



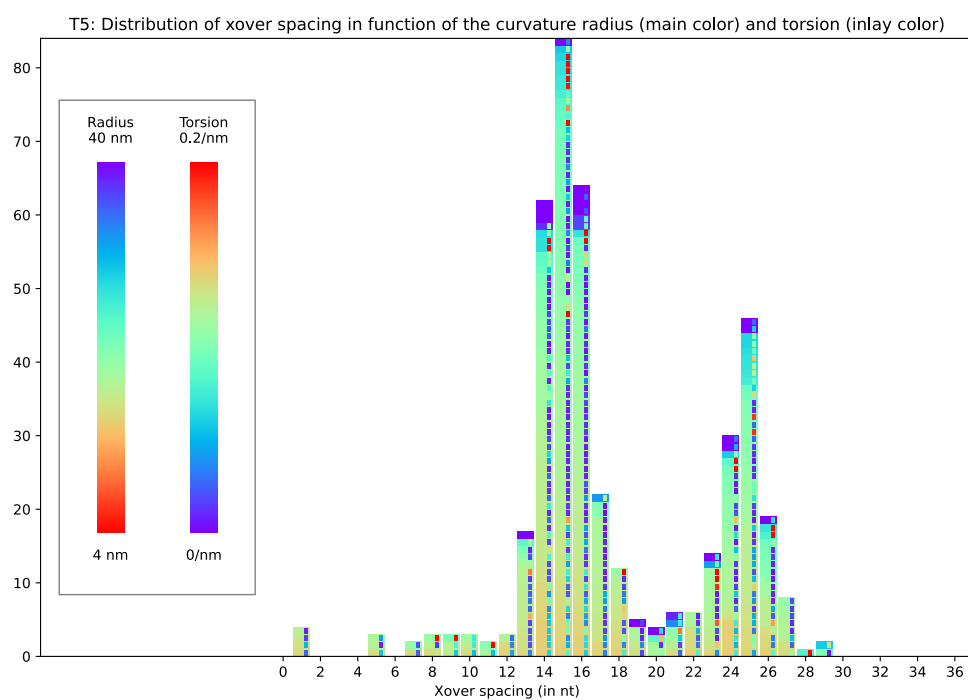


Figure S140: Distribution of staple strand crossover spacing in the T5-origami.

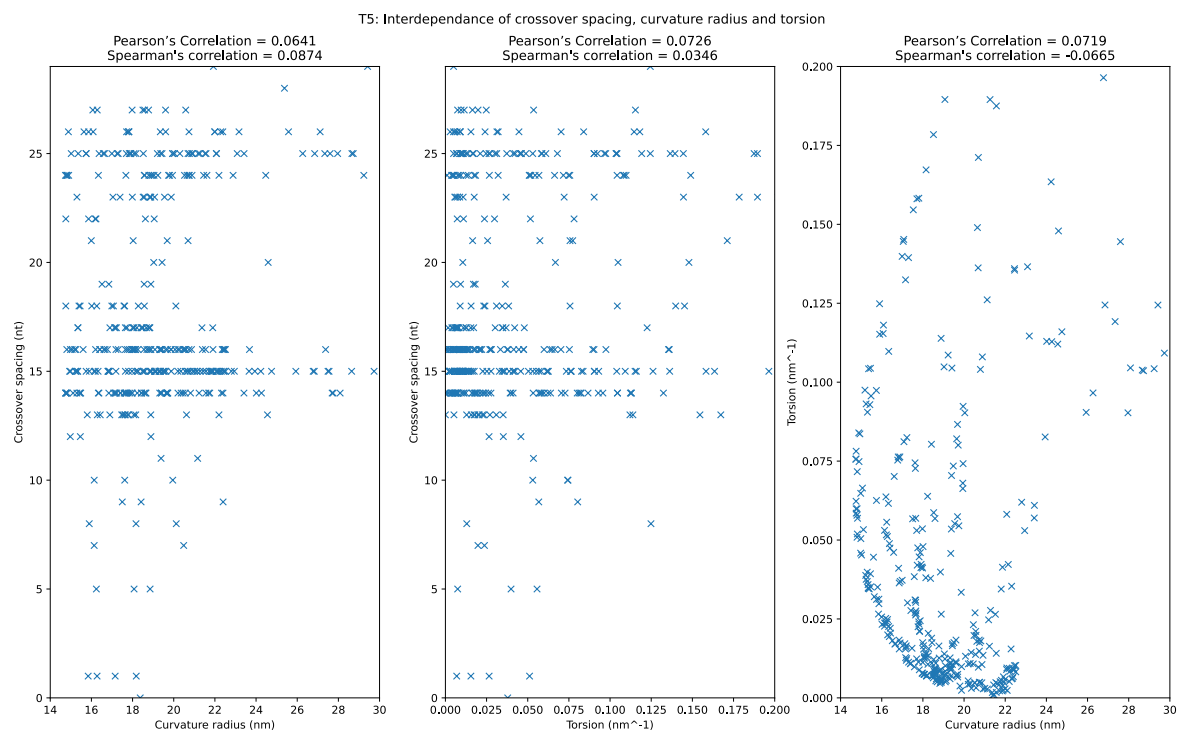


Figure S141: Evaluation of the correlations between crossover spacing, local curvature radius, and torsion in the T5-origami.

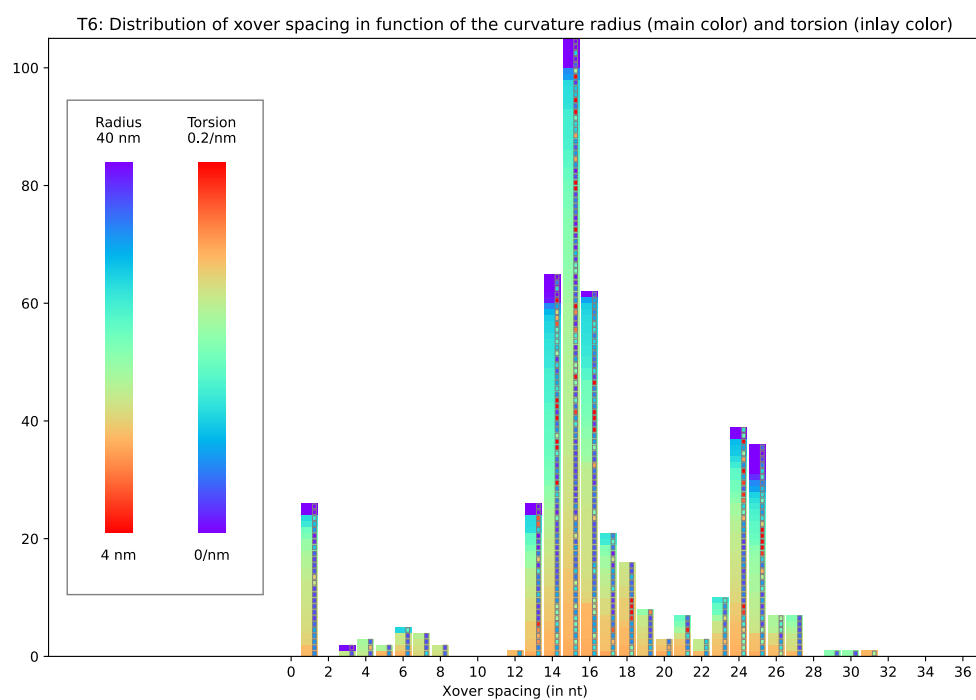


Figure S142: Distribution of staple strand crossover spacing correlated (in colors) with local curvature radius and torsion in the T6-origami.

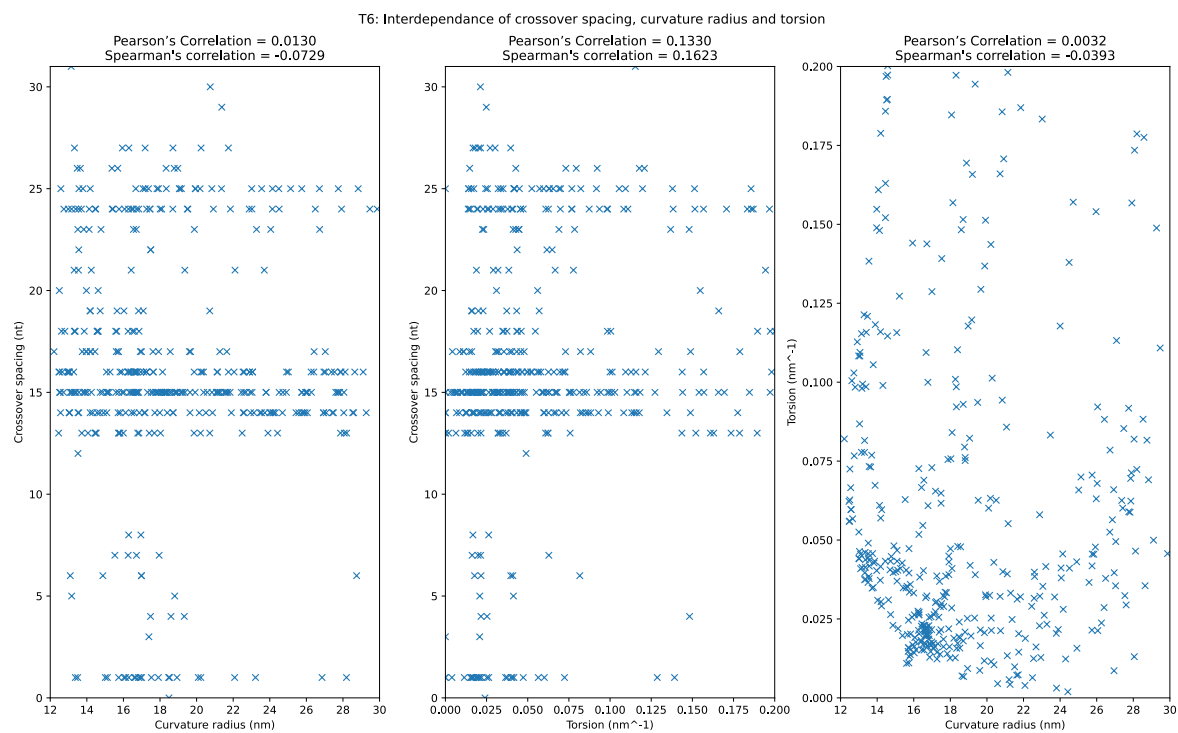


Figure S143: Evaluation of the correlations between crossover spacing, local curvature radius, and torsion in the T6-origami.

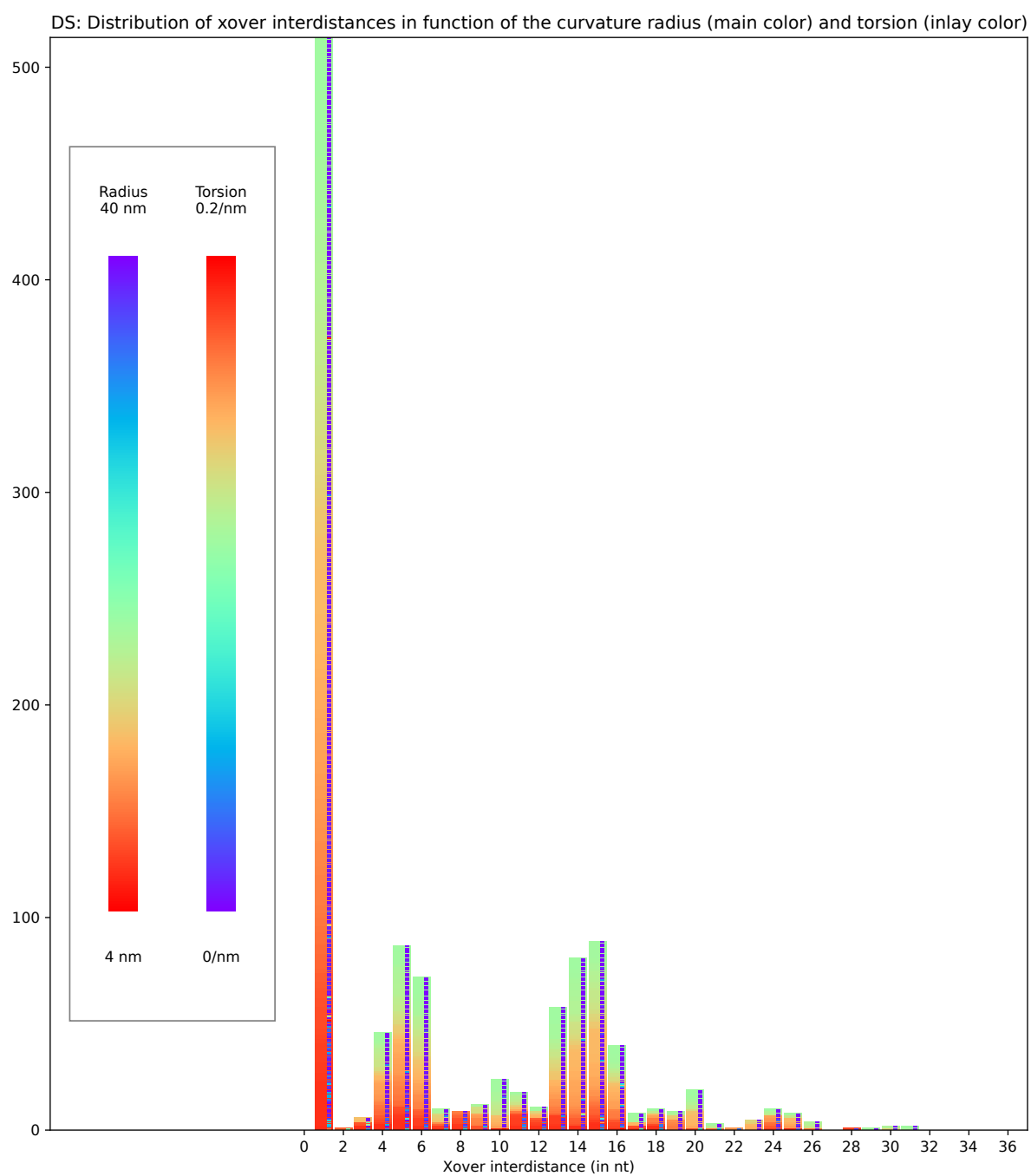


Figure S144: Distribution of staple strand crossover spacing correlated (in colors) with local curvature radius and torsion in the DS-origami.



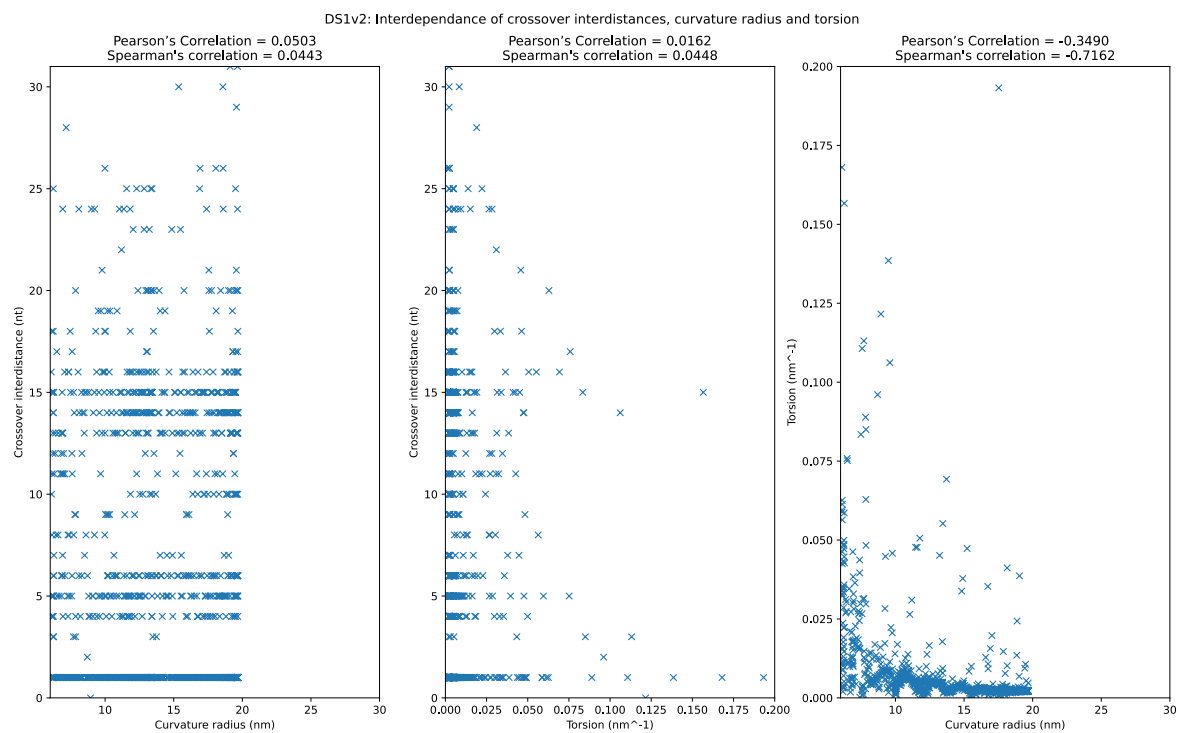


Figure S145: Evaluation of the correlations between crossover spacing, local curvature radius, and torsion in the DS-origami.

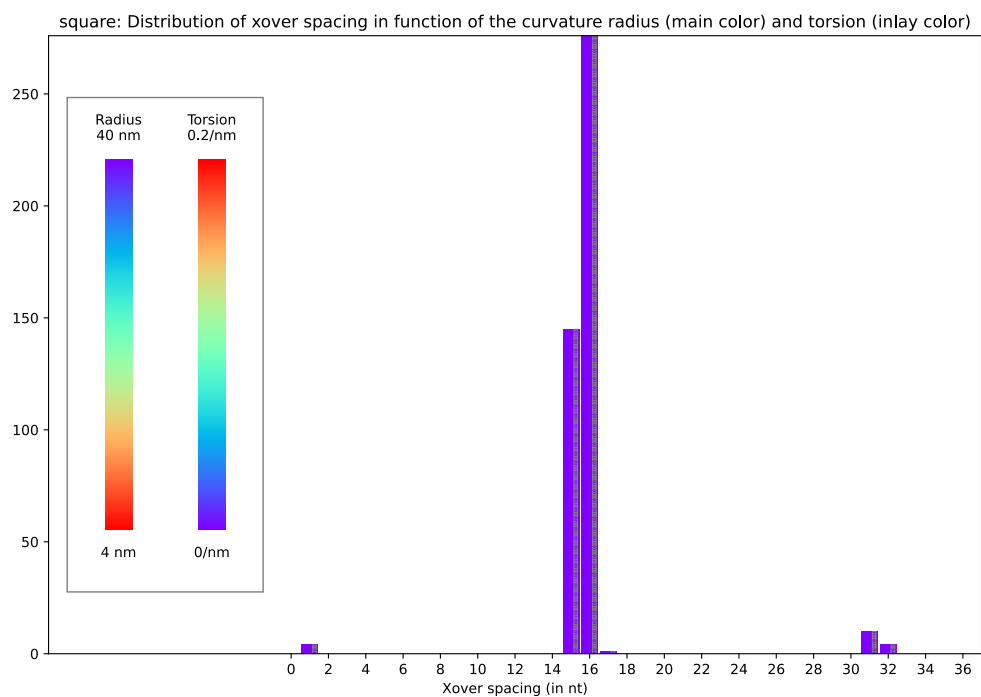
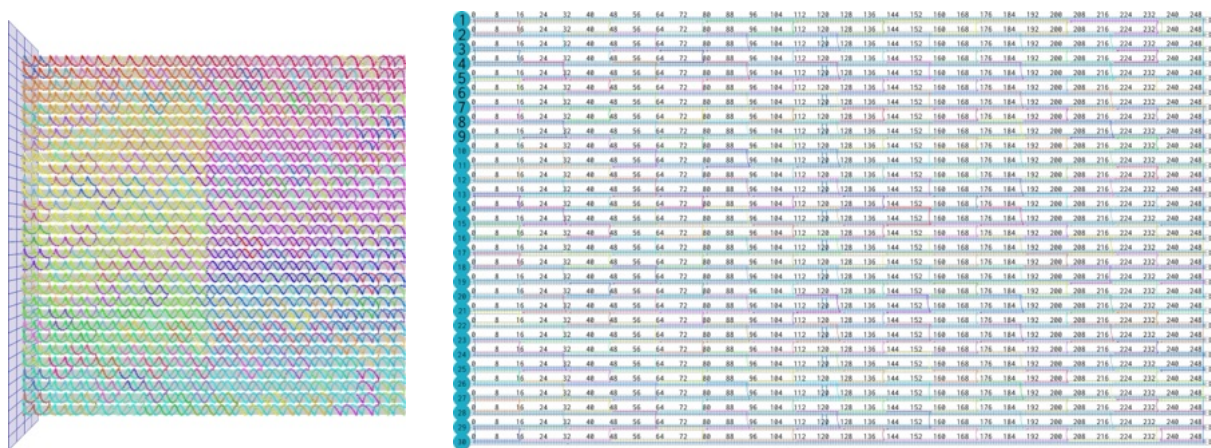


Figure S146: Distribution of staple strand crossover spacing correlated (in colors) with local curvature radius and torsion in a classic 30-helix square origami (7560-nt scaffold), corresponding to the design shown above.