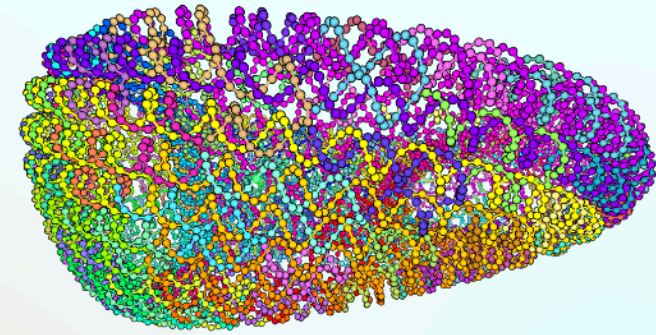
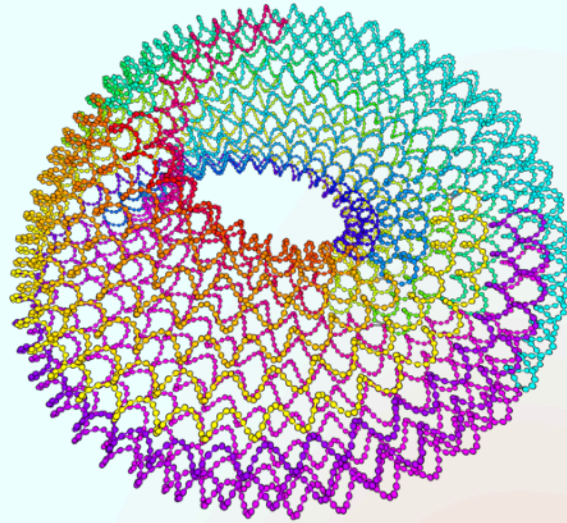
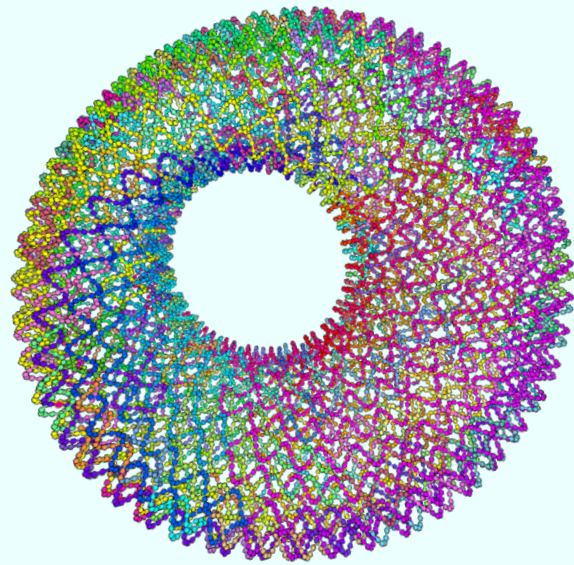
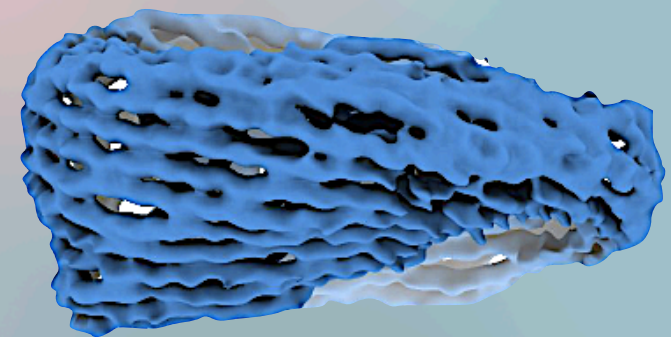
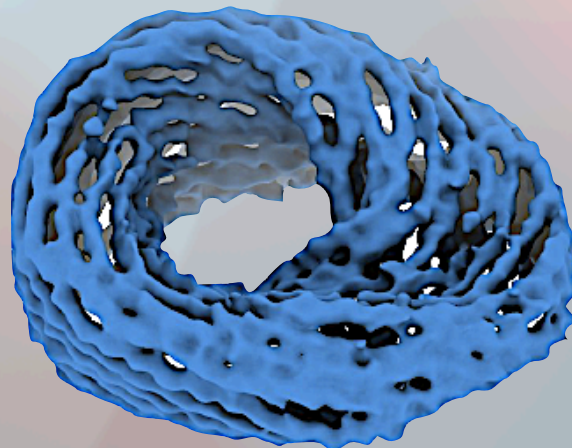
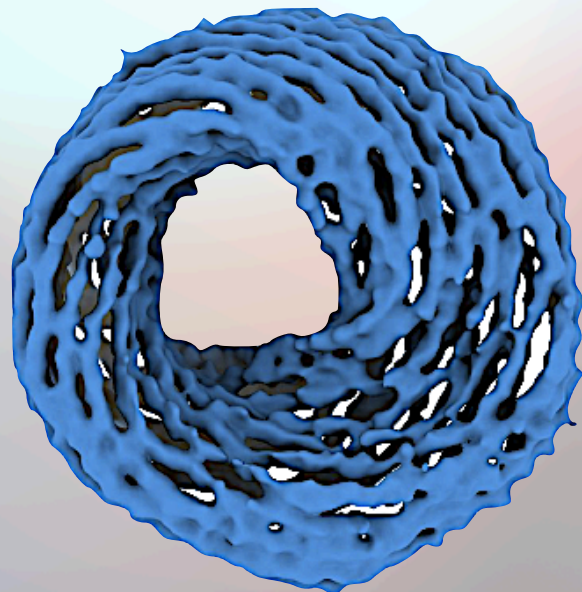




How do we make DNA origami with nano

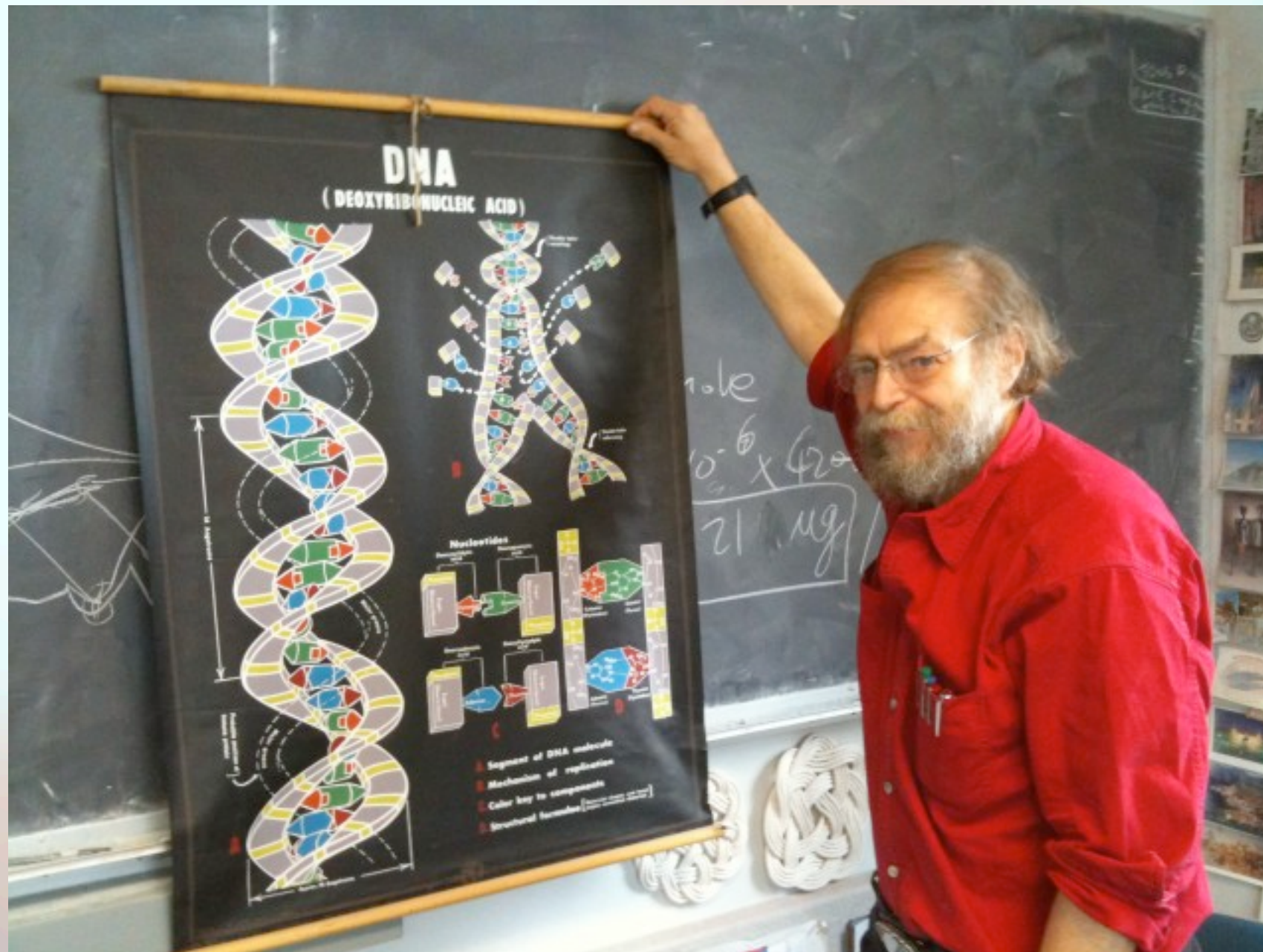
Nicolas Schabanel CNRS, LIP & IXXI, ÉNS de Lyon

Joint work with N. Levy, J. Finkel, A. Mills, P. Marcus, O. Hazard, D. Pchelina, J. Picot & G. Bellot



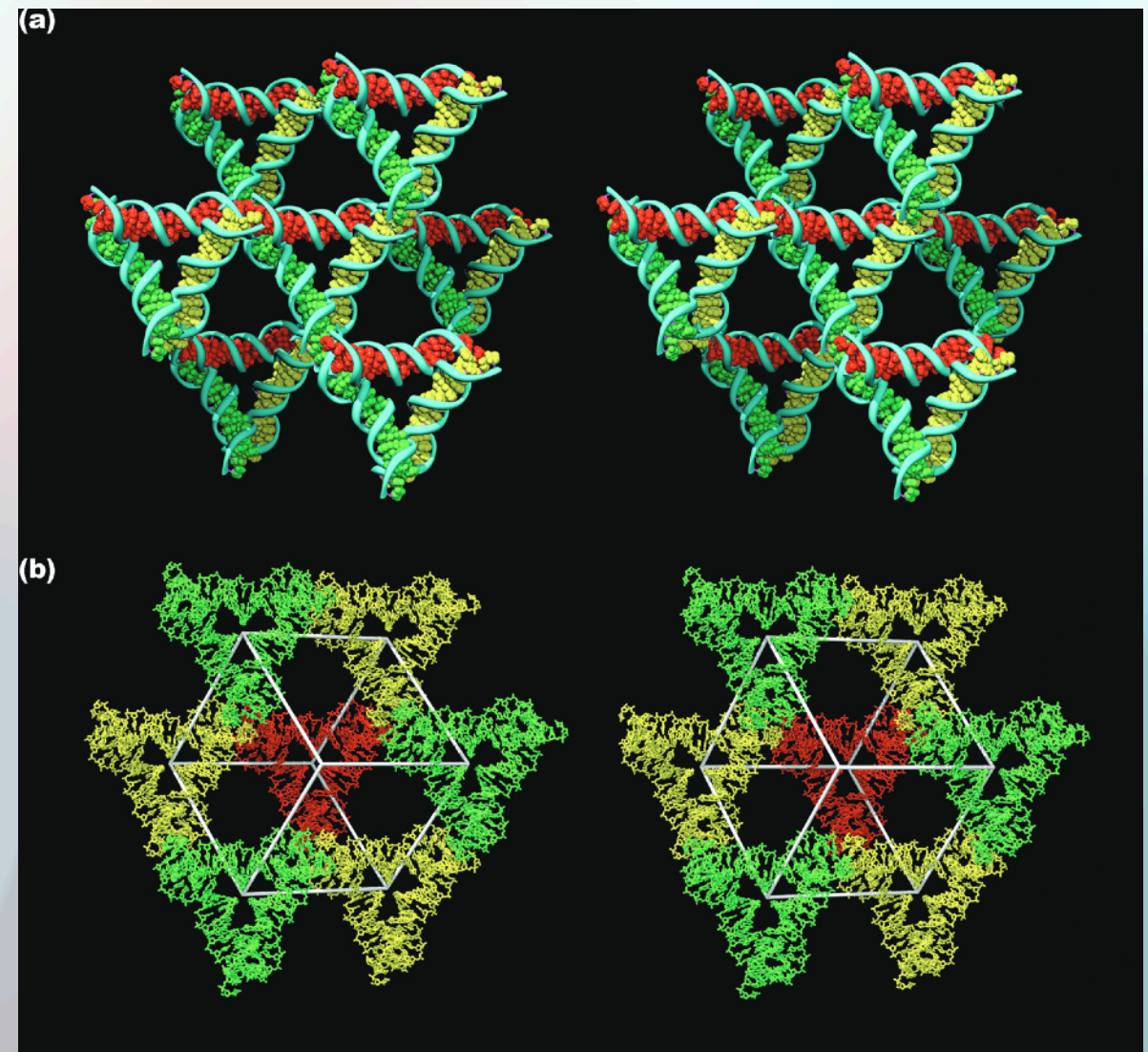
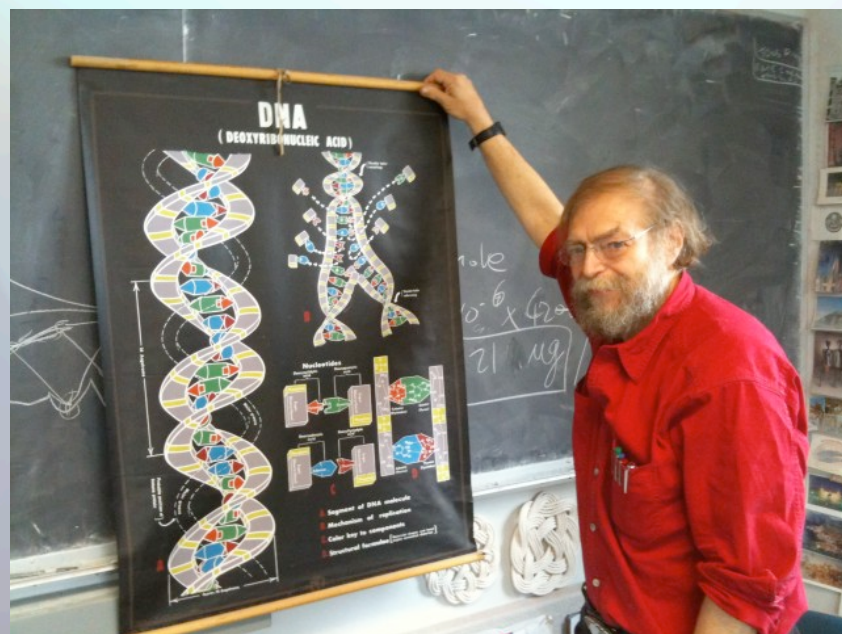
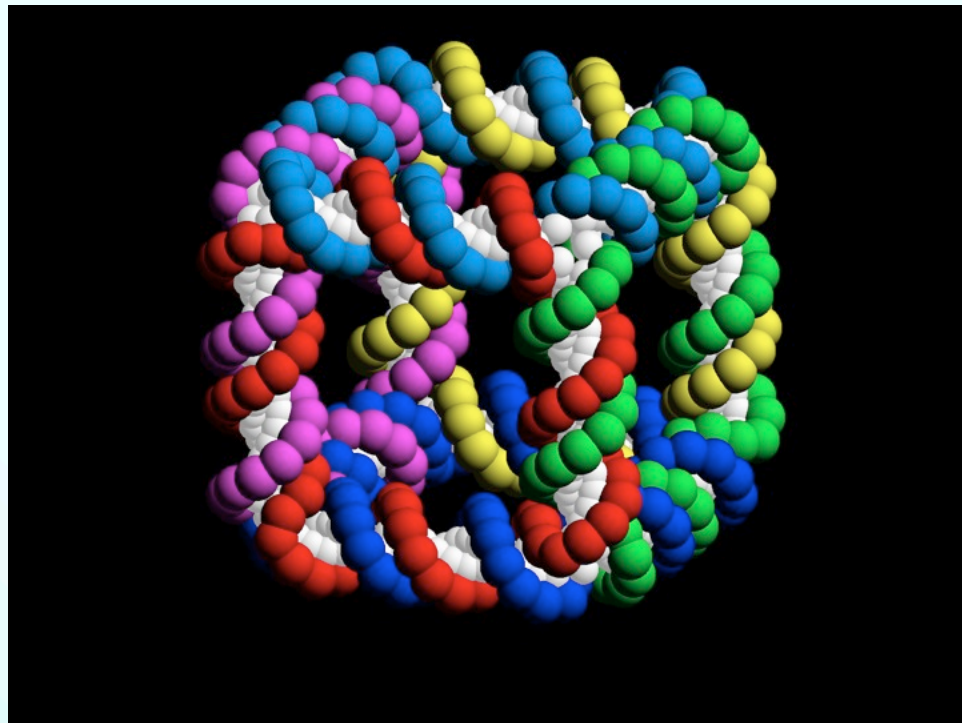
Using DNA to create shapes

Ned Seeman (1990s)



Using DNA to create shapes

Ned Seeman (1990s)

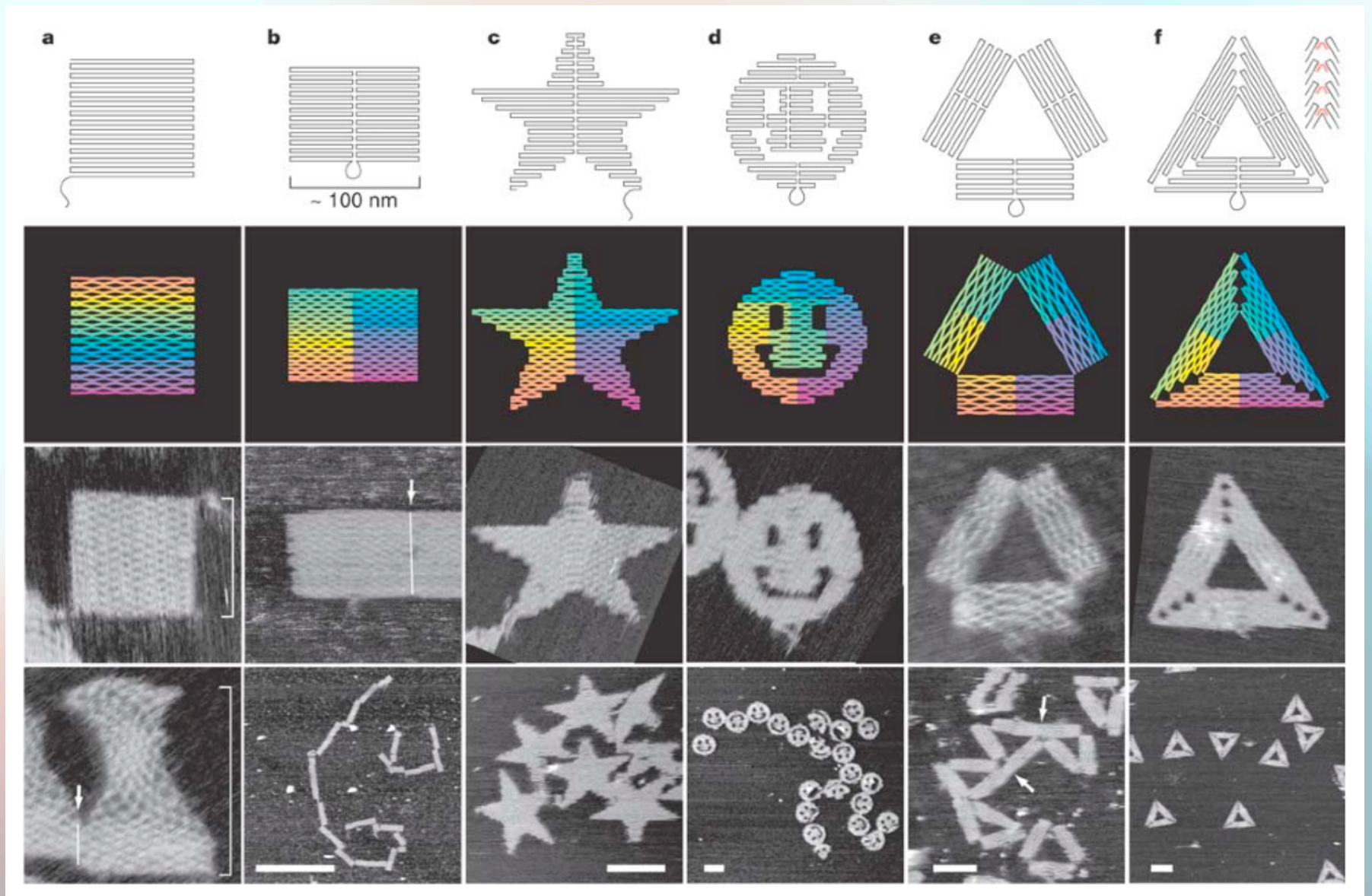


Create complementary strands inducing particular shapes

DNA origami



Paul Rothemund (2006)

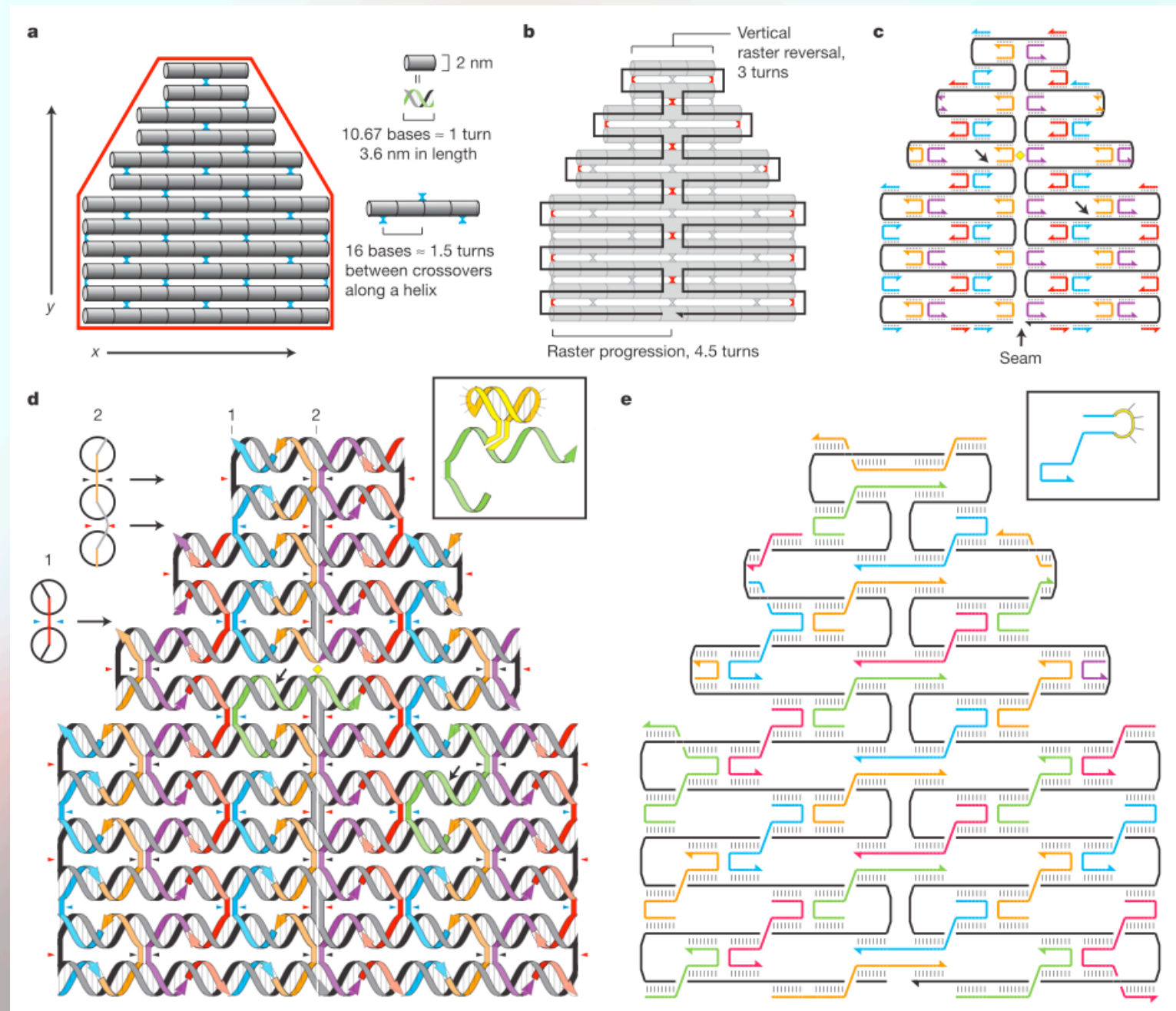


DNA origami

DNA double helix persistence length is $\sim 45\text{nm}$, that is ~ 135 base pairs (bp) $\rightarrow$ can be abstracted as cylinder with diameter 2nm

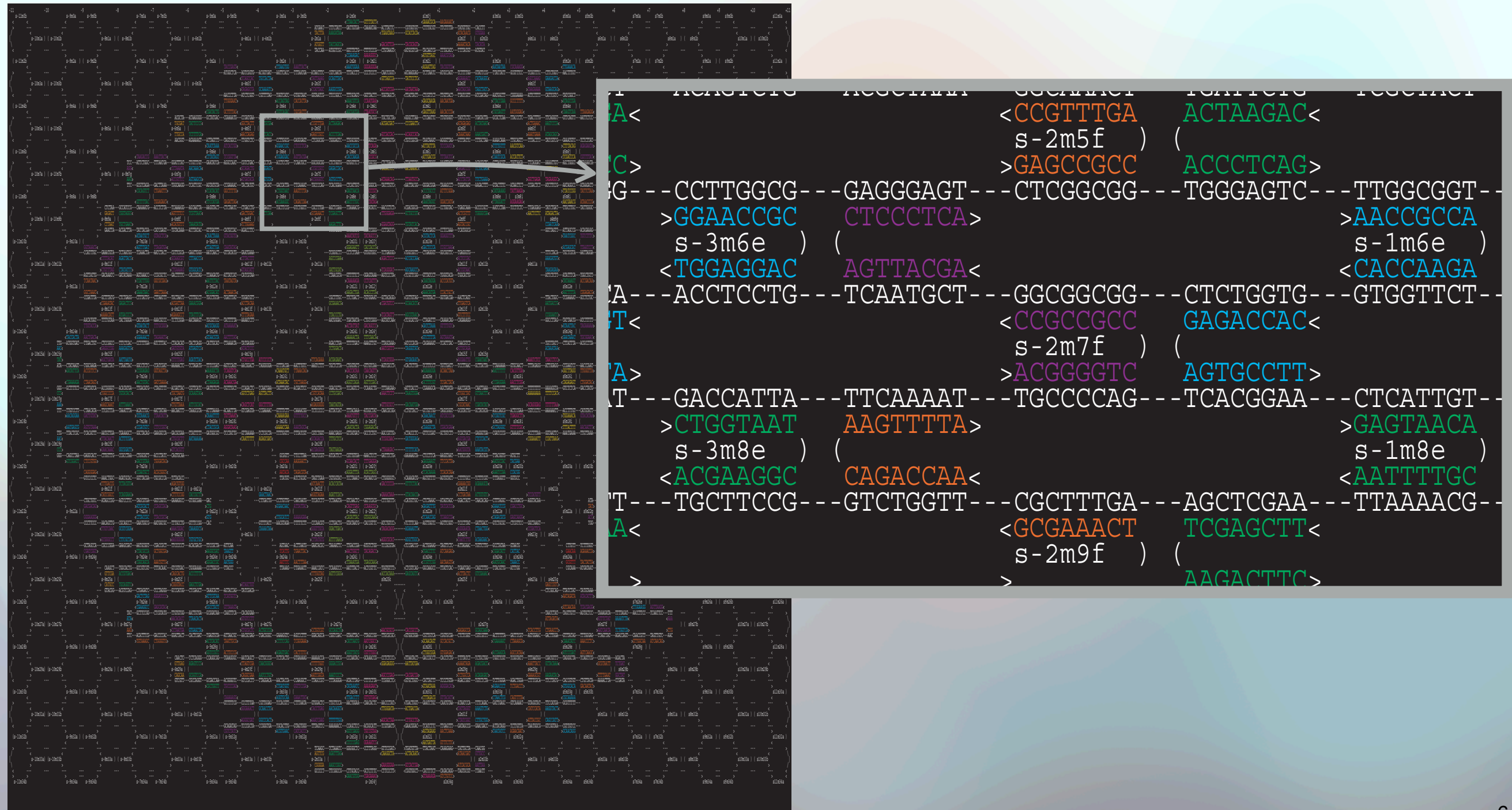


Paul Rothemund (2006)



The DNA of a virus is folded by the staples!

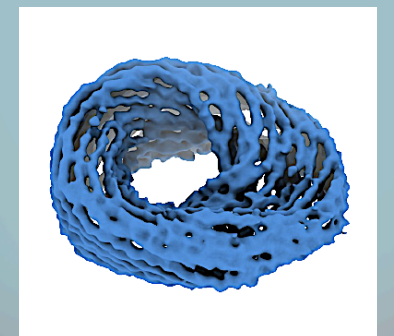
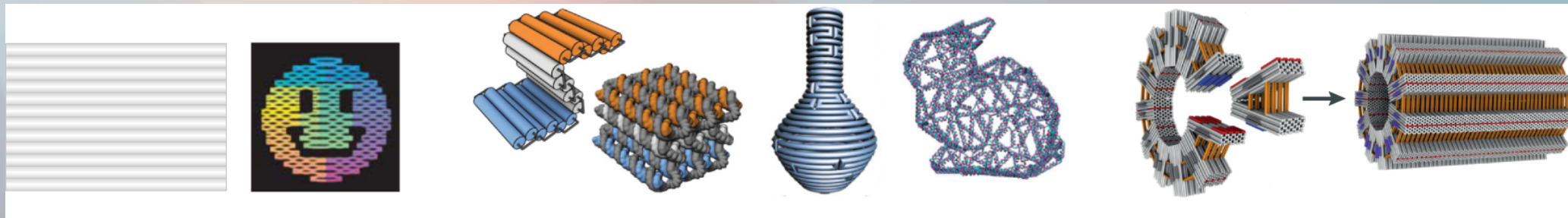
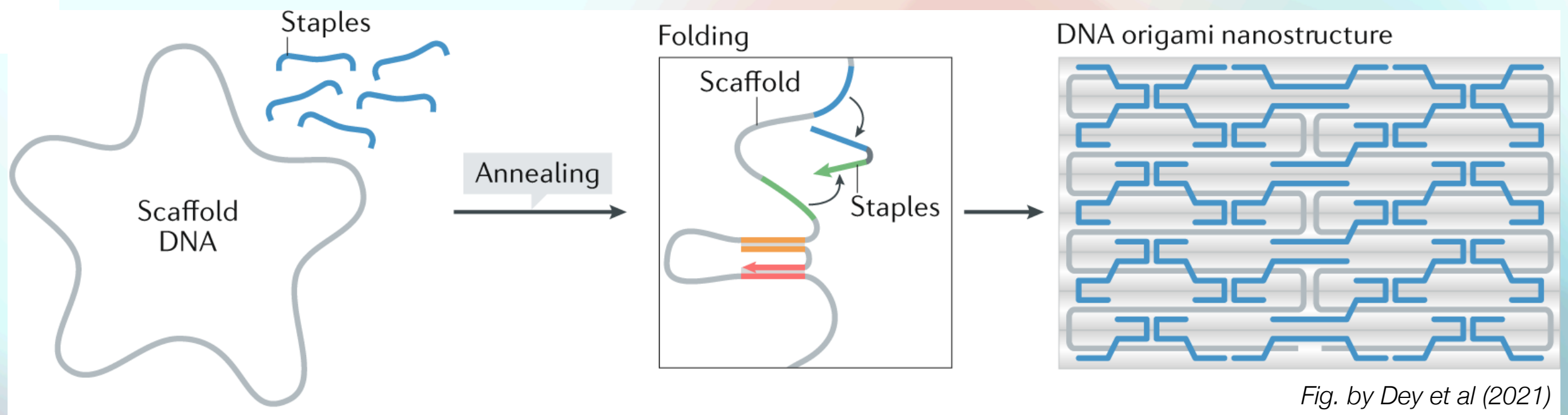
DNA origami



DNA origami

Annealing: assembly takes place when DNA mix cools down from 60°C to 40°C

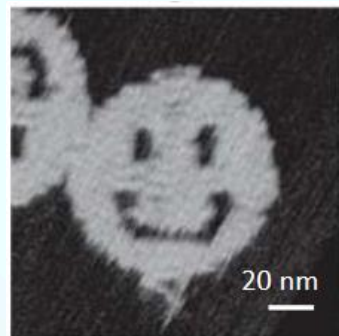
1. T° drops **fast** from 90°C to 65°C and then
2. T° decreases **slowly** from 65°C to 40°C (typically at -1°C/10min up to -1°C/2h)



DNA origami

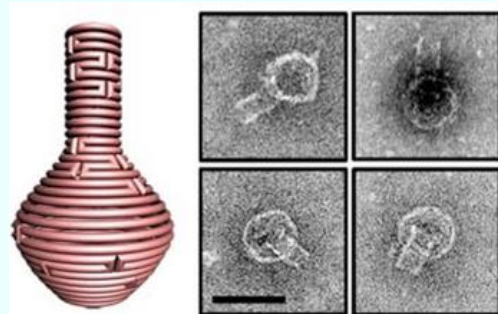
Some emblematic designs

Rothemund



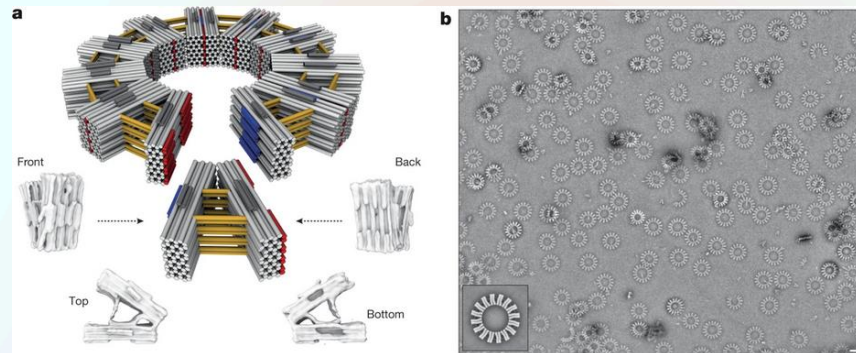
2006

Han *et al*



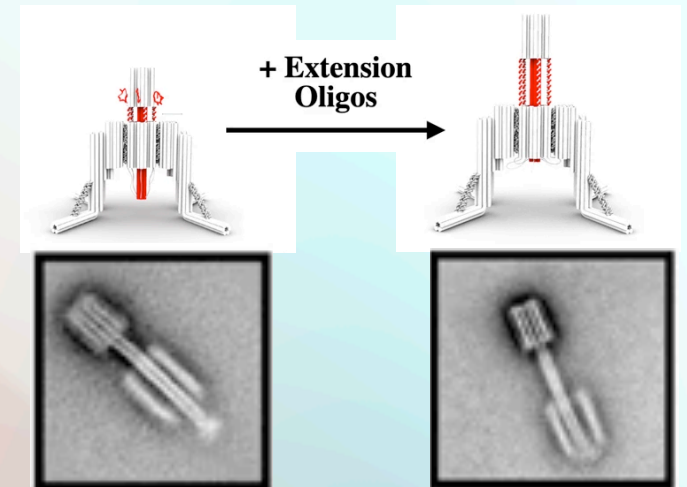
2011

Wagenbauer *et al*



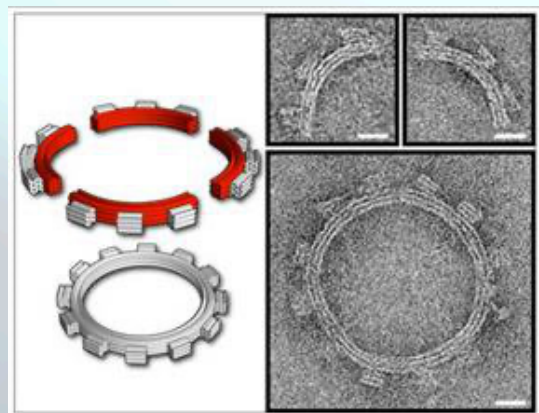
2017

Mills *et al*



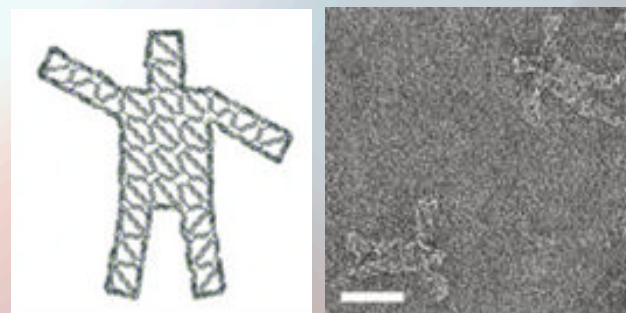
2023

2009



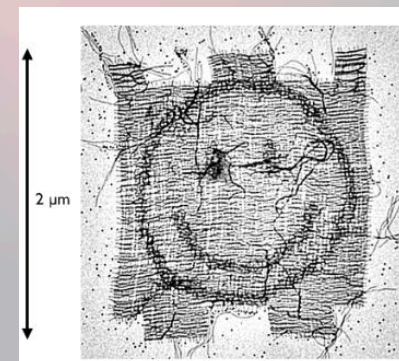
Dietz *et al*

2015



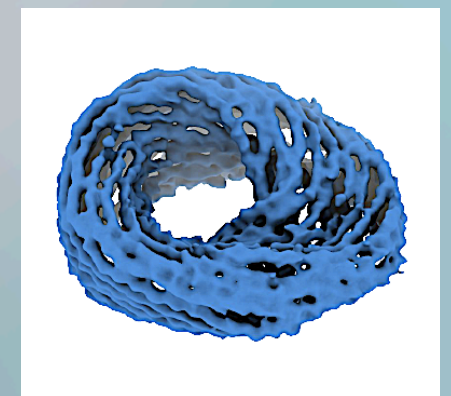
Benson *et al*

2021



Wintersinger *et al*

2022

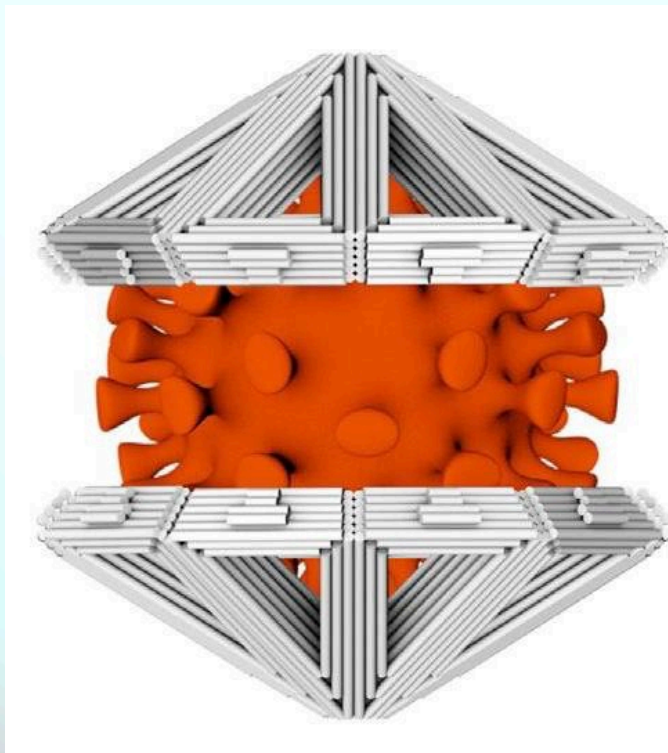


Levy, Finkel,
Mills, Bellot,
Schabanel

DNA origami

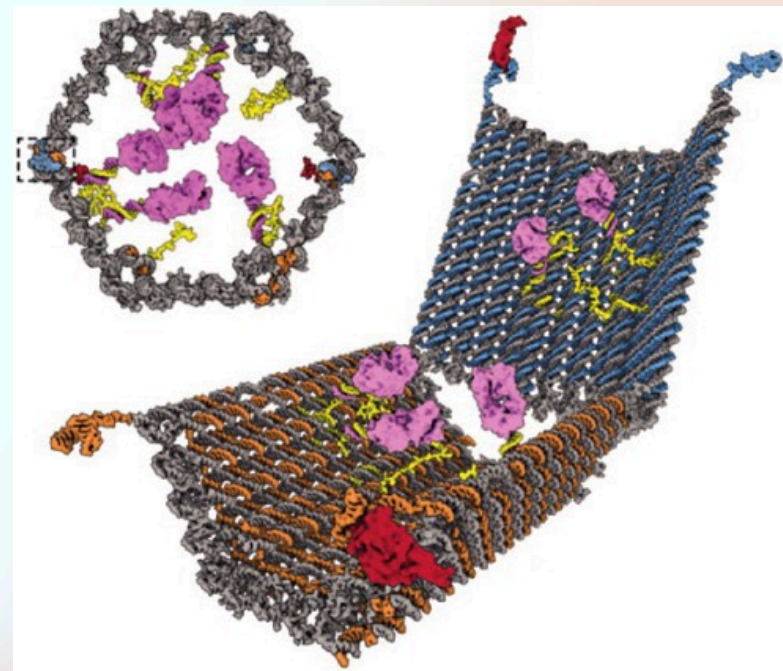
DNA Origami has many applications

In medicine



Traps for large viruses
Monferrer et al., 2023

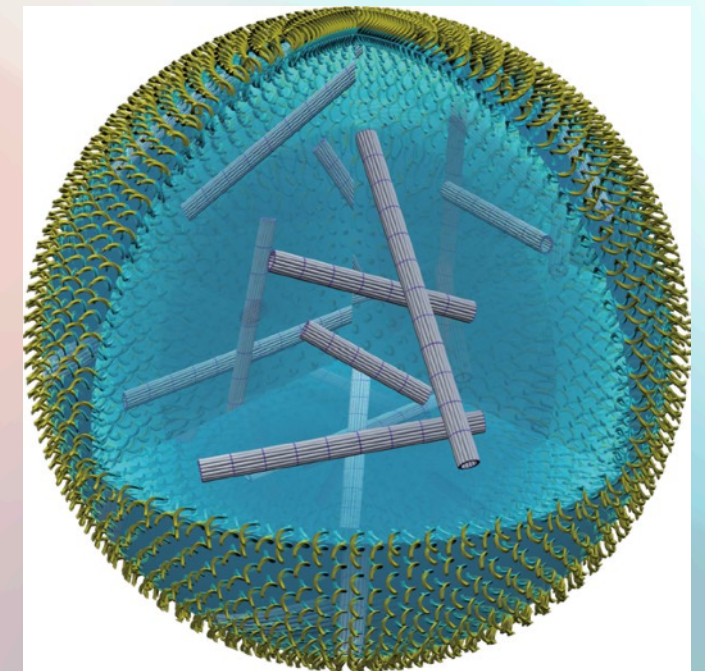
- Biosensor
- Nanomaterials



Targeted delivery of antibodies
Douglas et al., 2012

- Smart molecular devices
- Study of protein interactions, ...

In synthetic biology



Functional synthetic cytoskeleton
Zhan et al., 2022

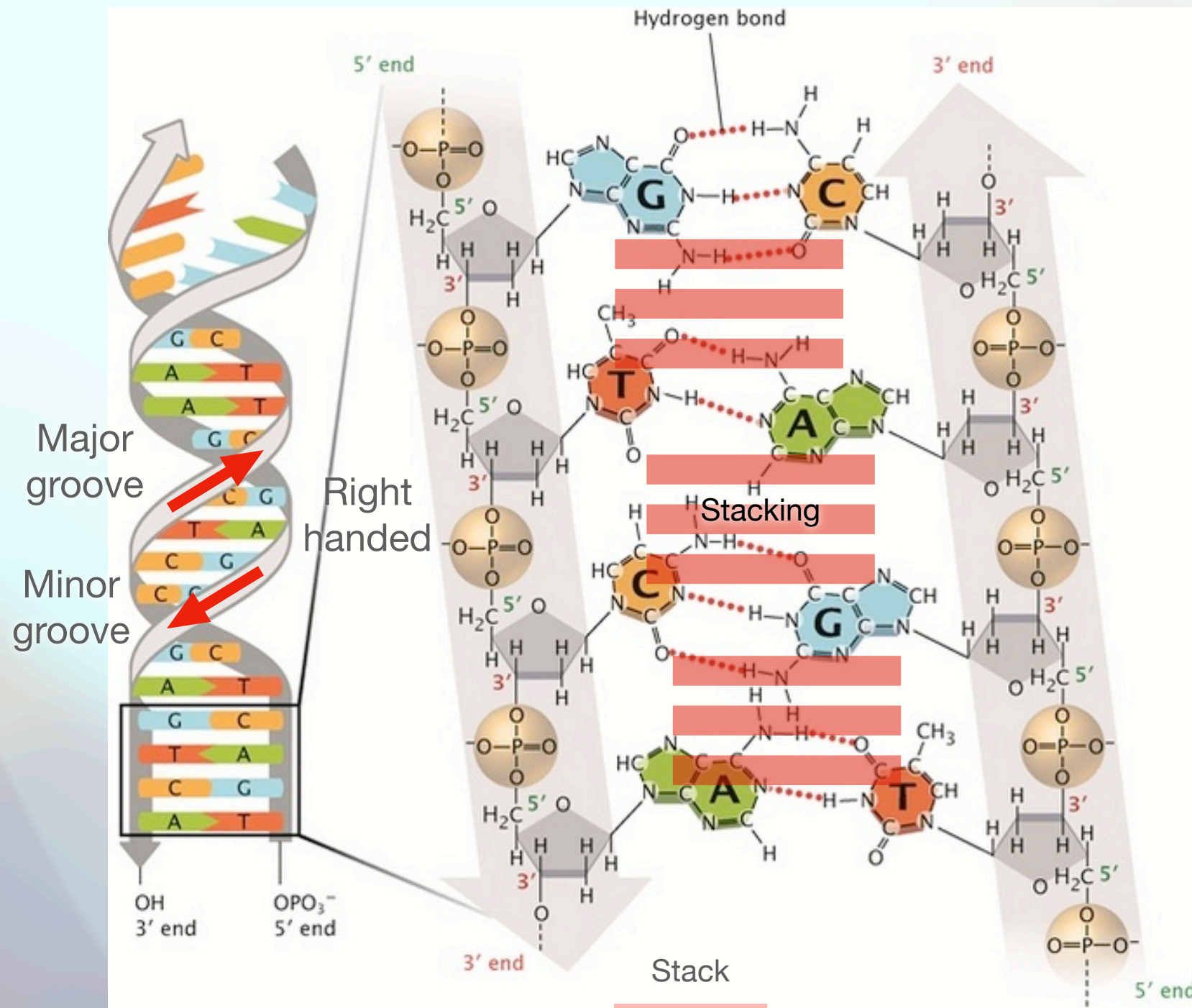
Only few crosslinks are enough even for curved shapes



What about curved surfaces?

DNA double-helix

B-form



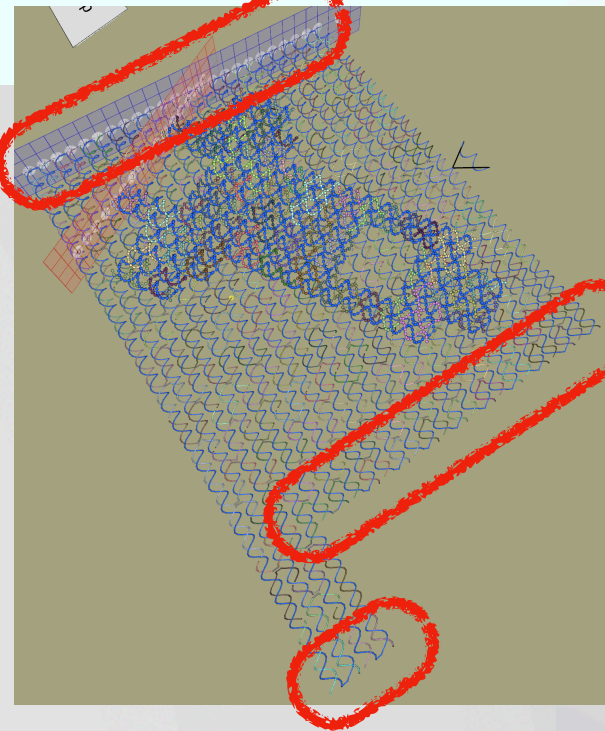
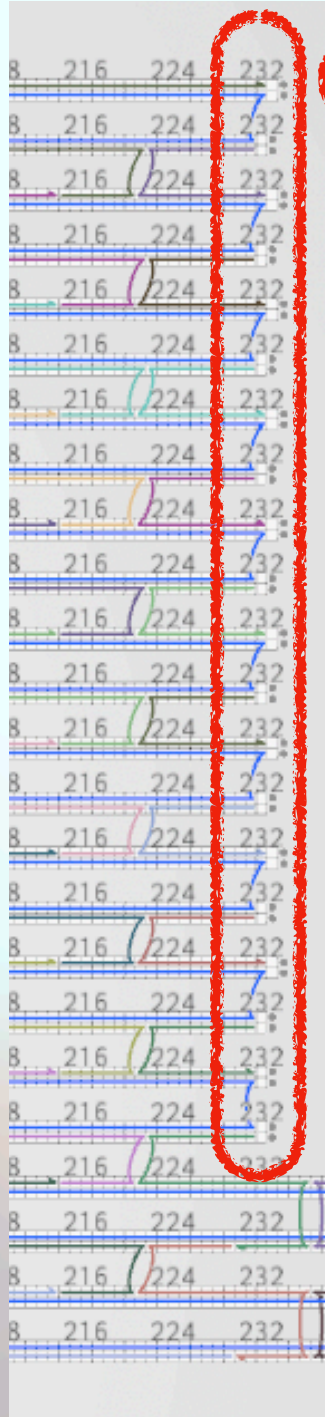
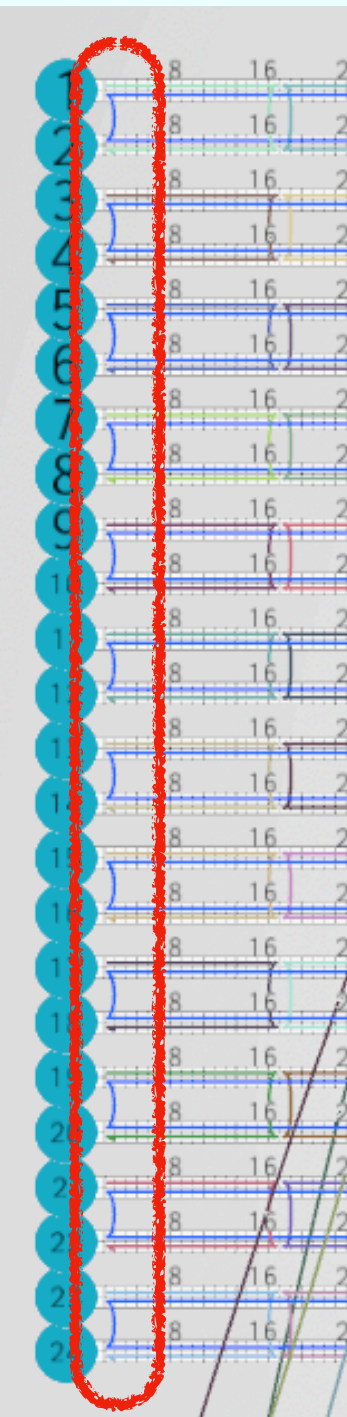
Major groove

Minor groove

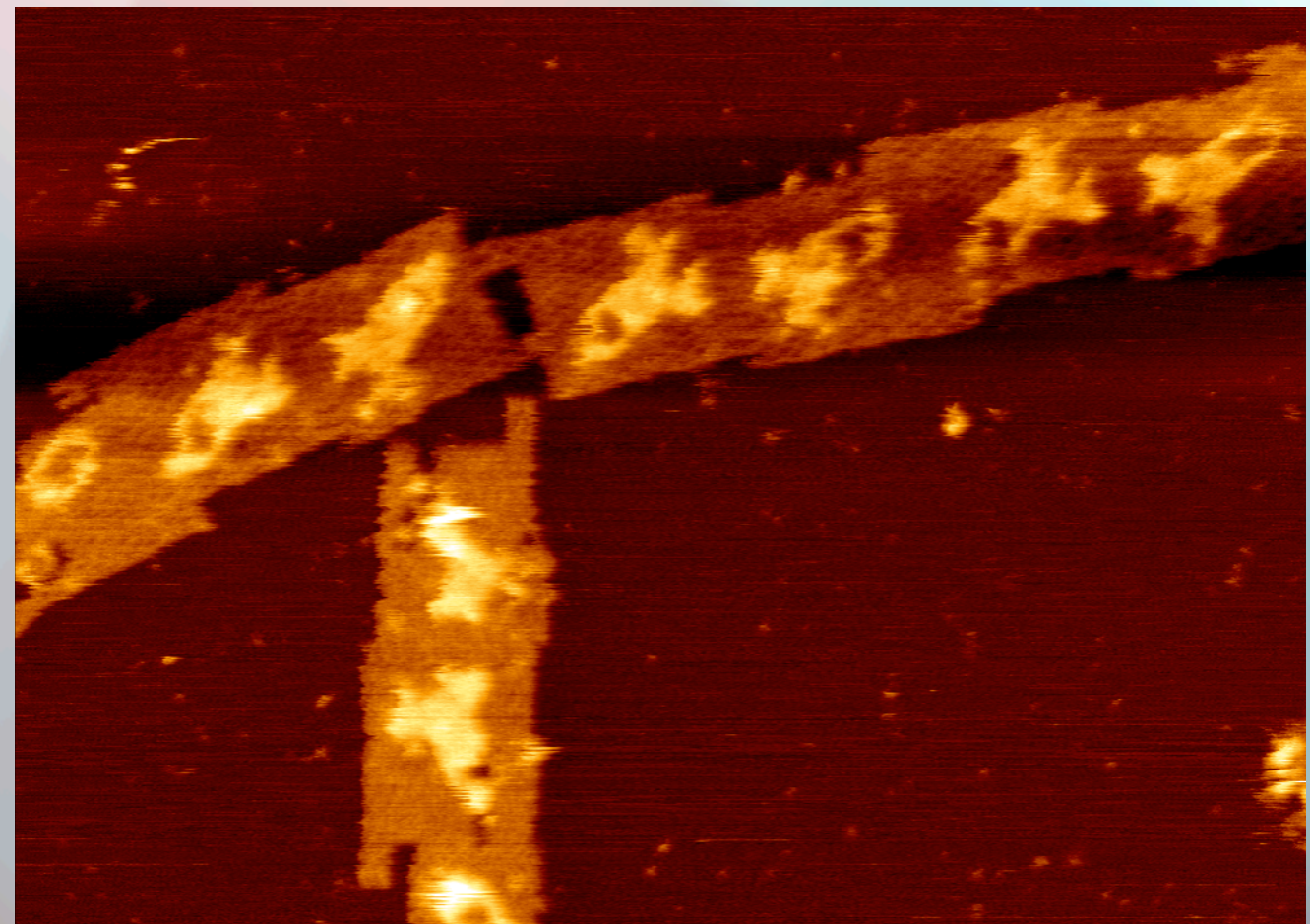
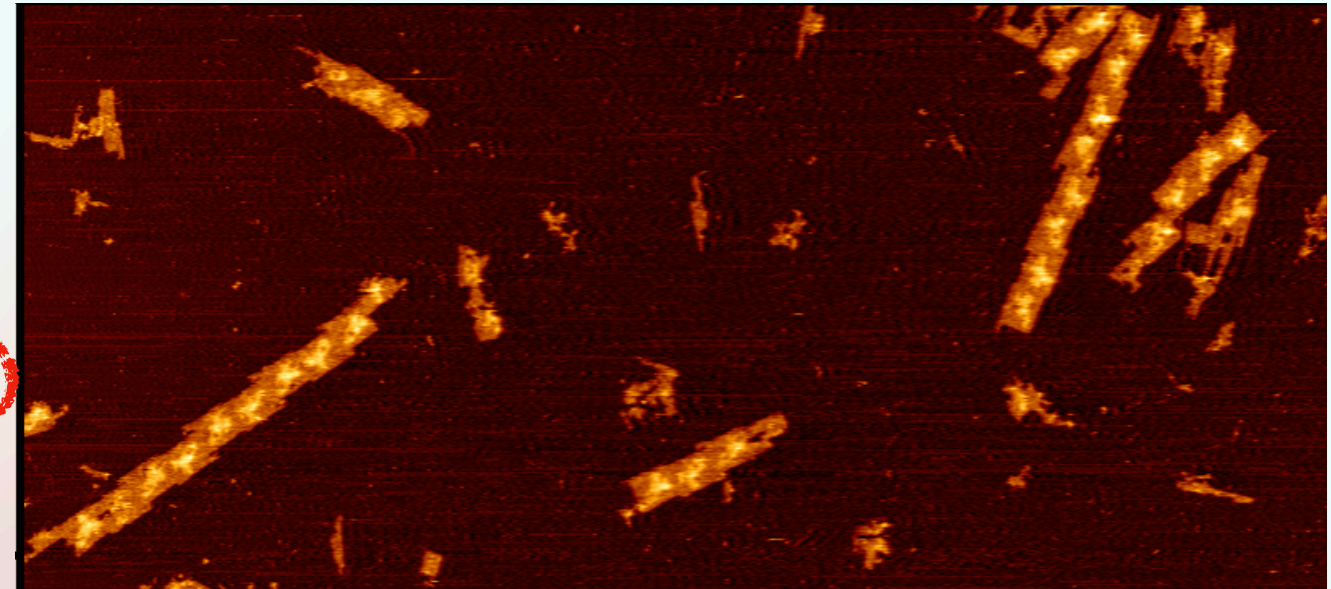
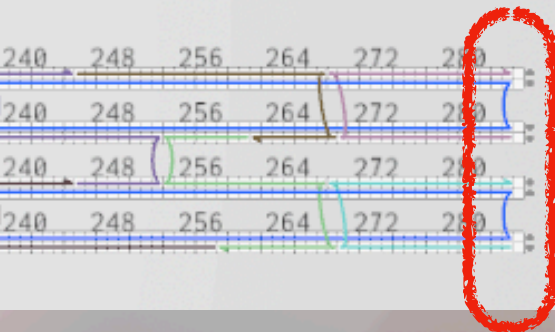
$$\Delta G^\circ = \Delta G^\circ(AT/GC) + \Delta G^\circ(GC/TA) + \Delta G^\circ(TA/CG) + \Delta G^\circ(CG/AT)$$

Bonding energy

Stacking phenomenon

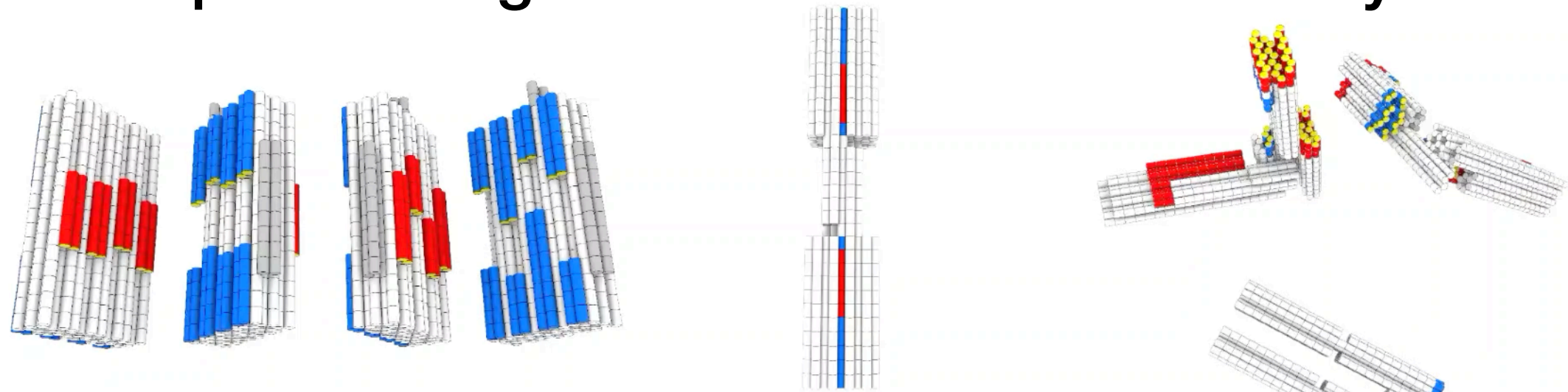
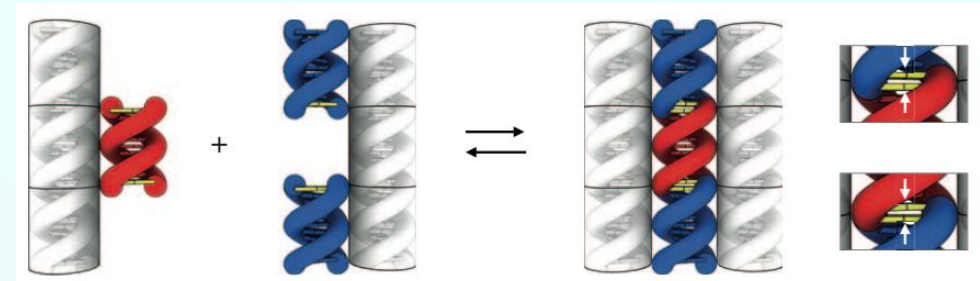


**Blunt ends
yields
stacking**



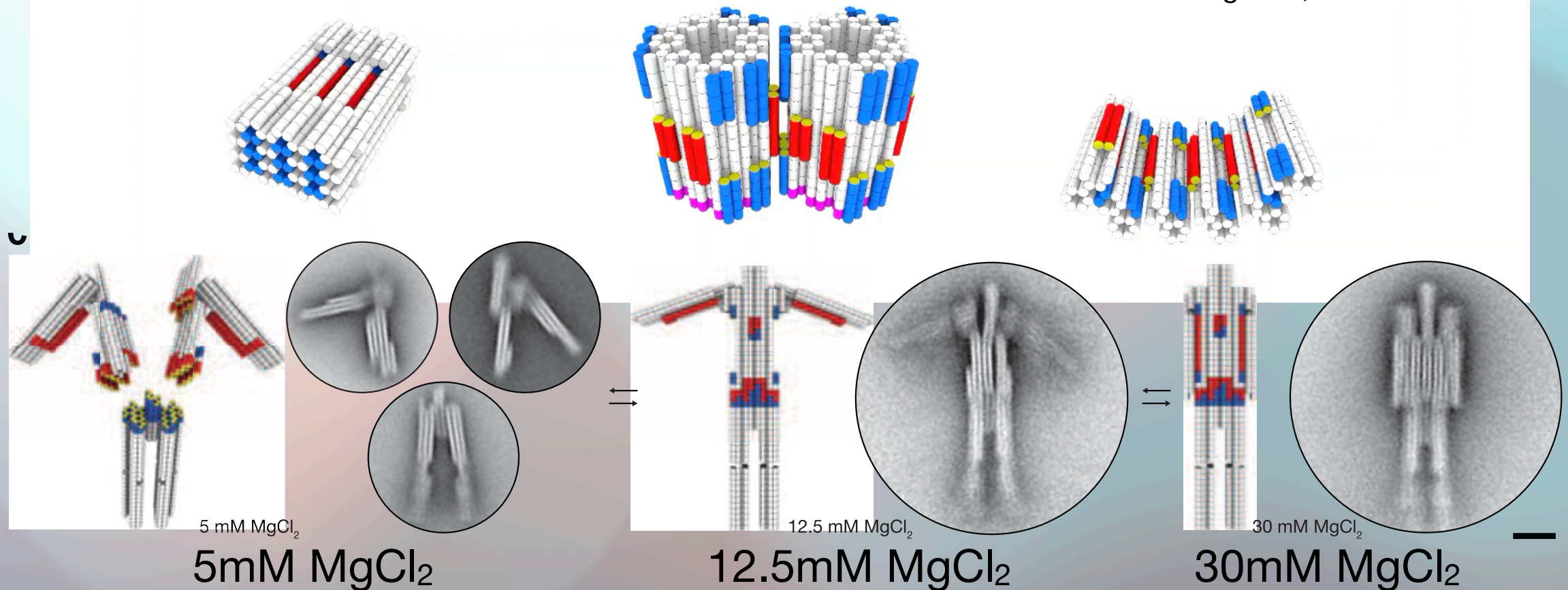
Using stacking

Multicomponent origamis — Reversible assembly



Changing Mg^{2+} concentration allows to bind multi-components using stacking interactions

Gerling et al, Science 2015



5 mM MgCl_2

5mM MgCl_2

12.5 mM MgCl_2

12.5mM MgCl_2

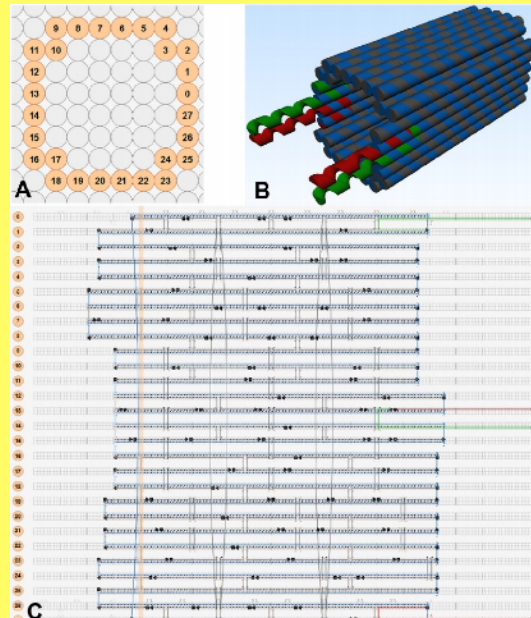
30 mM MgCl_2

30mM MgCl_2

Preexisting softwares

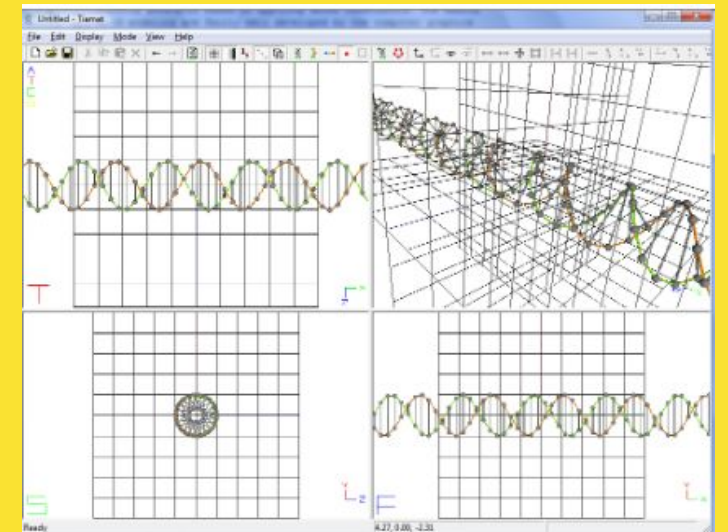
Abstract 2D representation

- ▶ Cadnano (2009)
- ▶ Scadnano (2019)
- ▶ **Efficient 2D view editing**



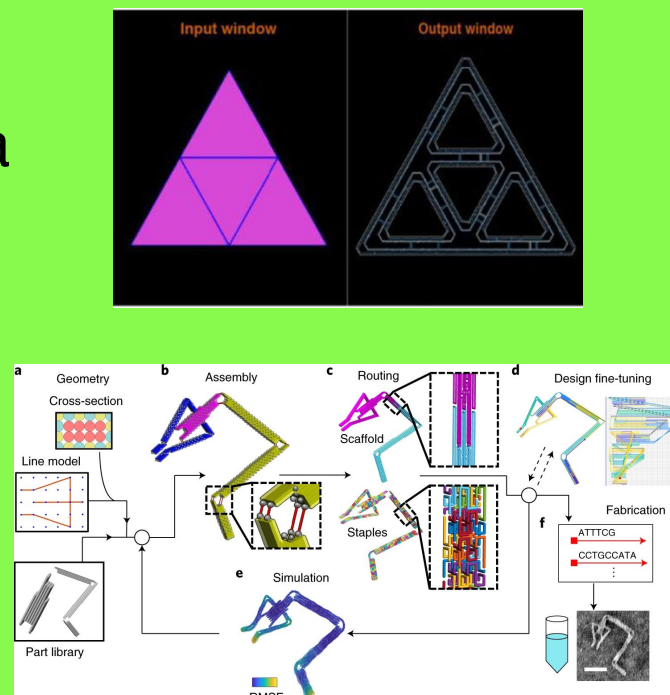
3D view & editing

- ▶ Tiamat (2009)
- ▶ vHelix (2014)
- ▶ adenita (2019)
- ▶ OxDNAviewer (2019)
- ▶ **Wysiwyg**



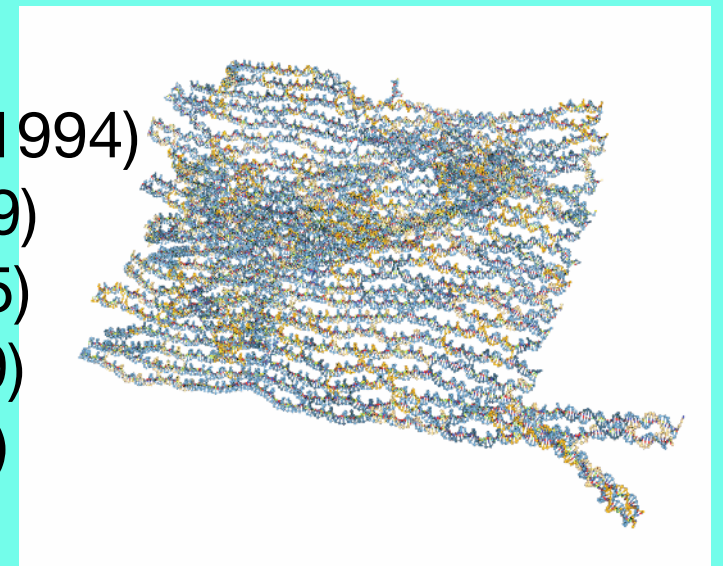
Automation

- ▶ Deadalus (2016)
- ▶ Perdrix, Talos, Athena (2019)
- ▶ MagicDNA (2021)
- ▶ **Auto-stapling**
- ▶ **Auto-scaffolding**
- ▶ **Articulated designs**



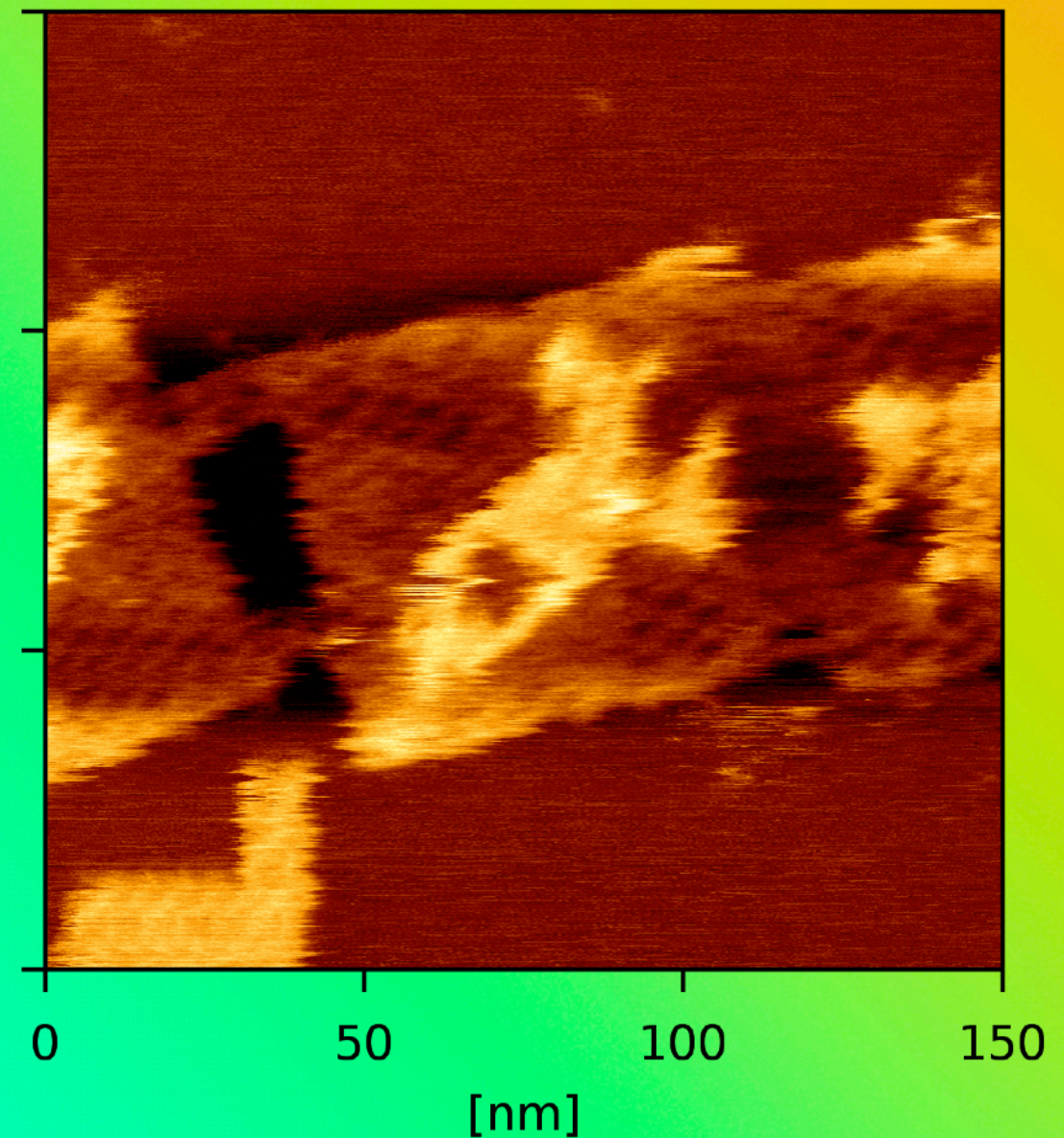
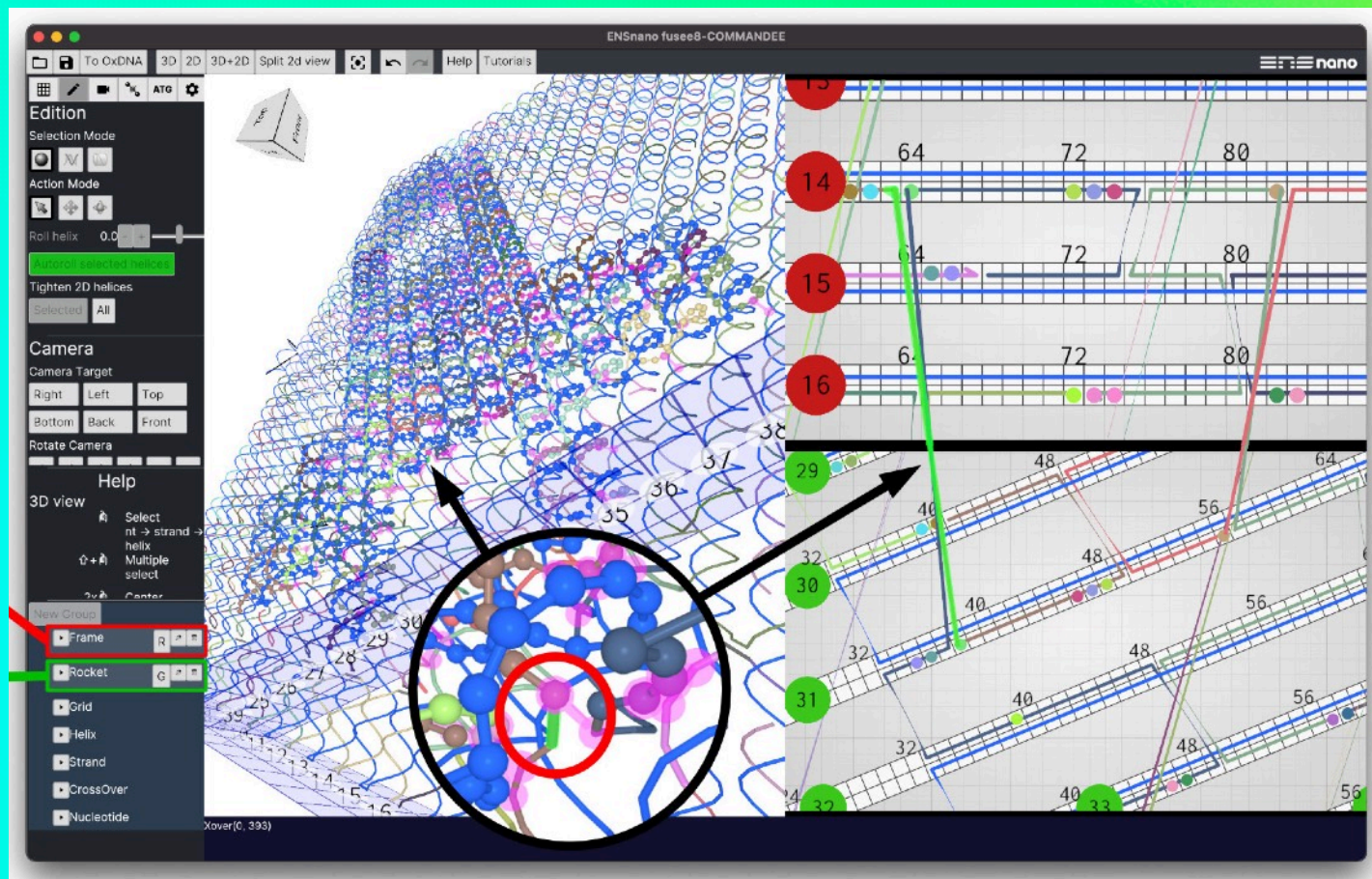
Physical simulation

- ▶ ViennaRNA (1994)
- ▶ Nupack (2009)
- ▶ OxDNA (2015)
- ▶ mrDNA (2019)
- ▶ SNUPI (2020)



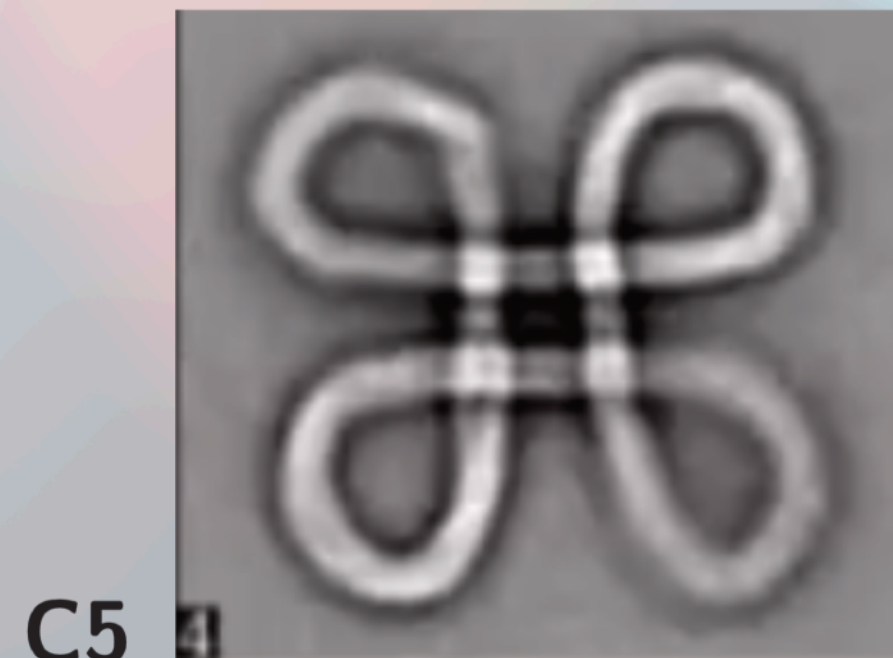
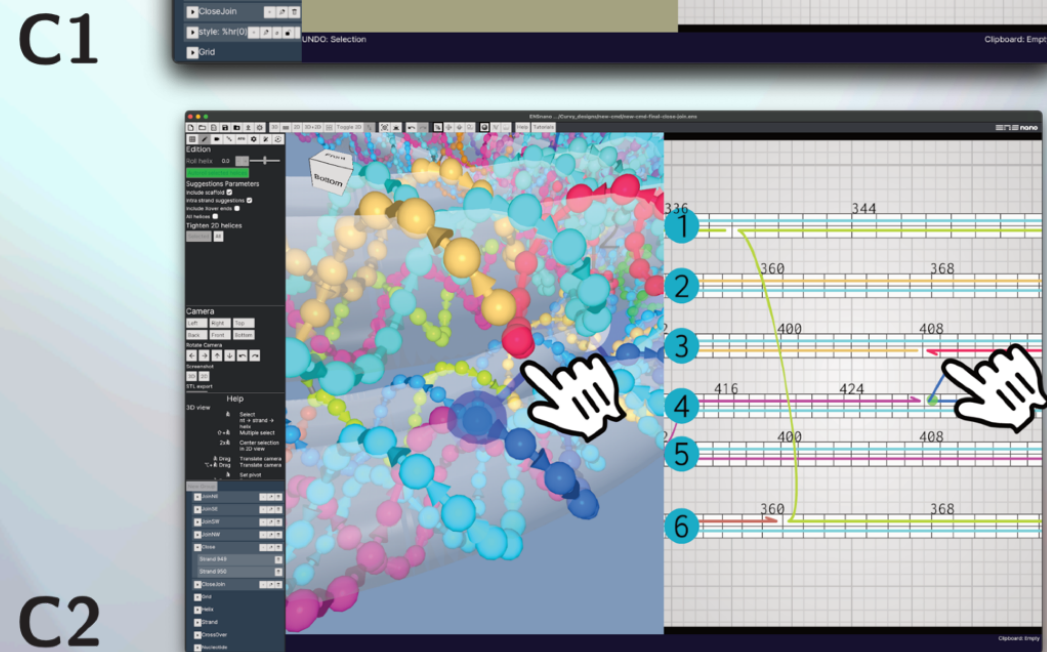
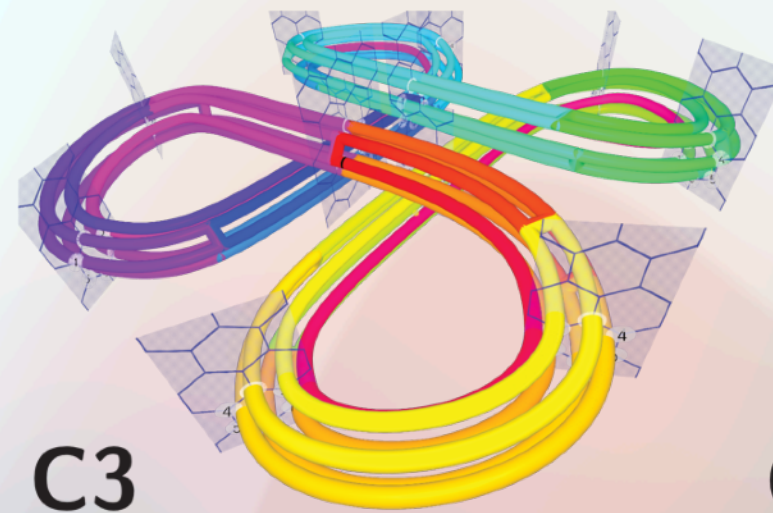
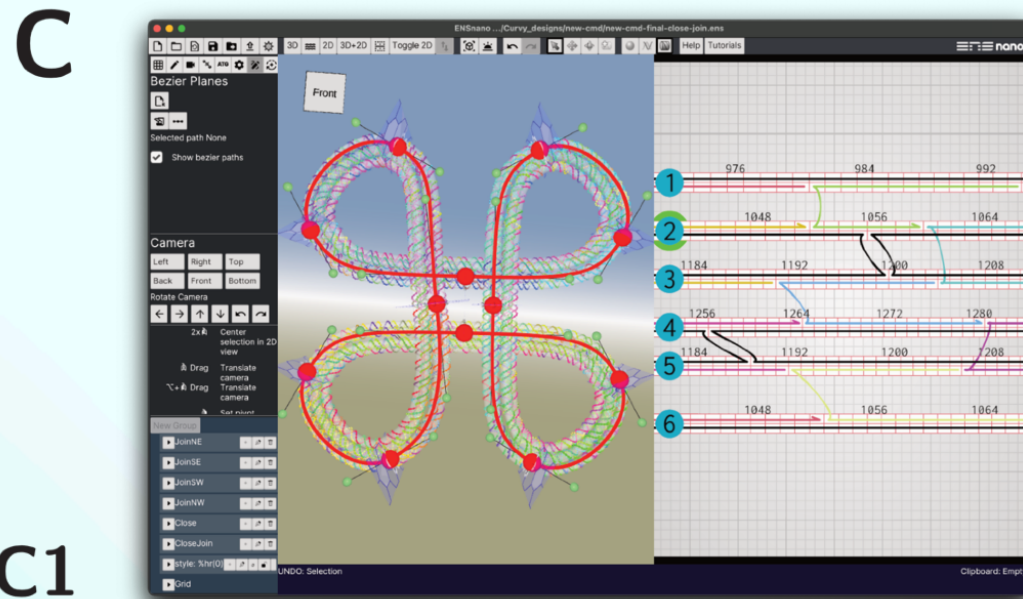
- ▶ **Feedbacks on designs**

Glimpse of DNA nanostructures made with nano



Glimpse of our DNA nanostructures

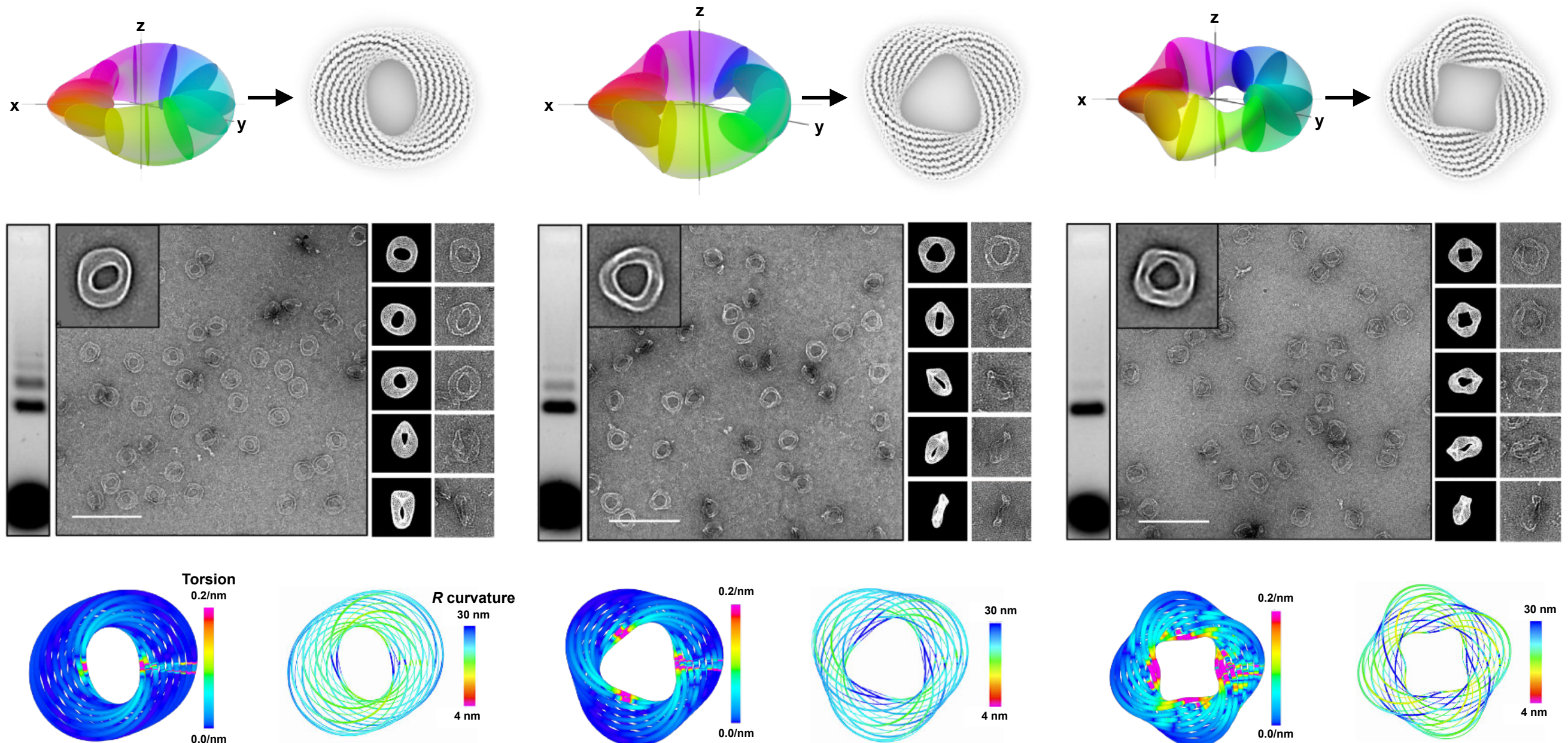
Experimental work with G. Bellot, J. Finkel and A. Mills



Diameter: 89 nm

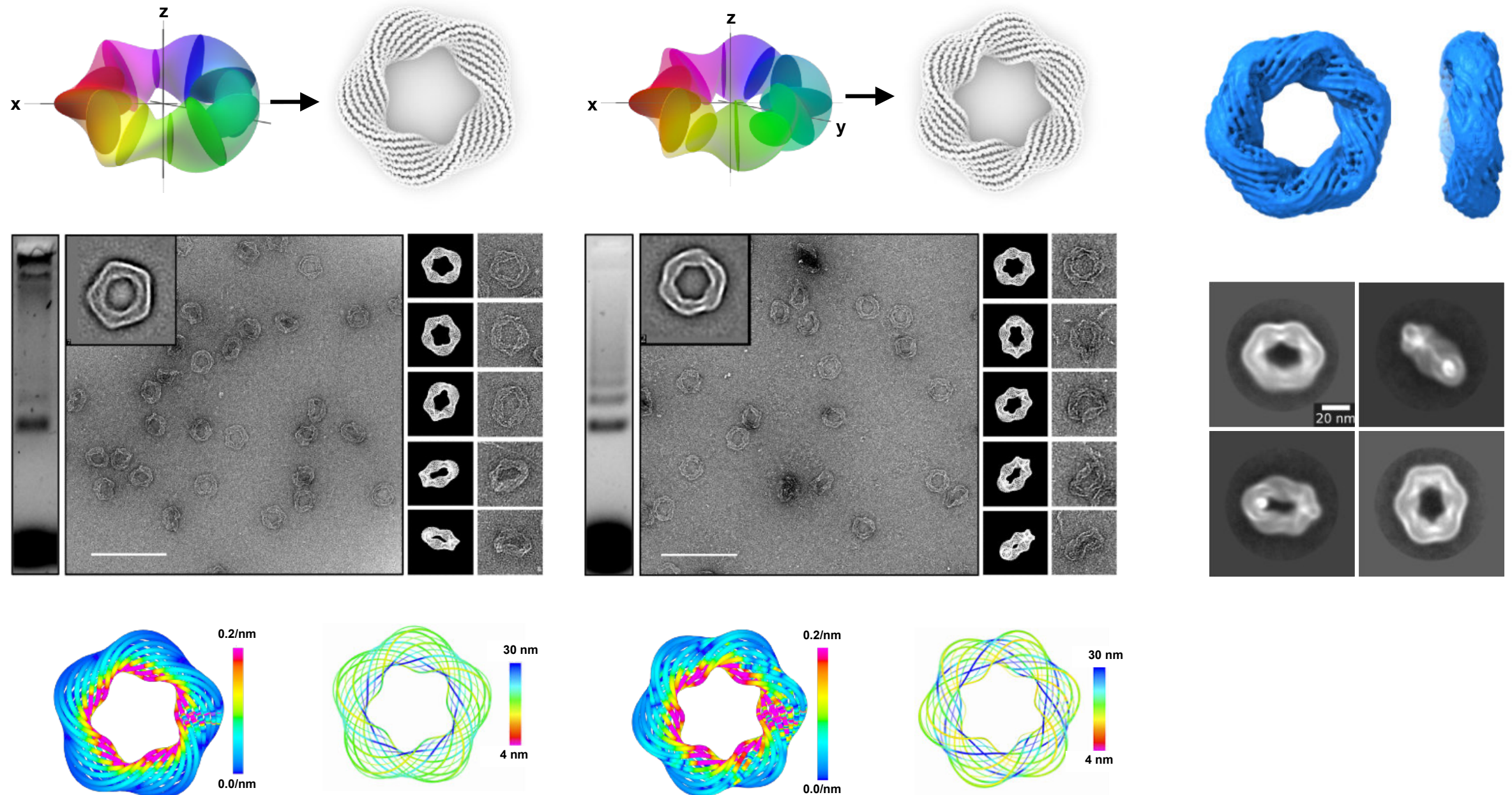
Glimpse of our DNA nanostructures

Experimental work with G. Bellot, J. Finkel and A. Mills



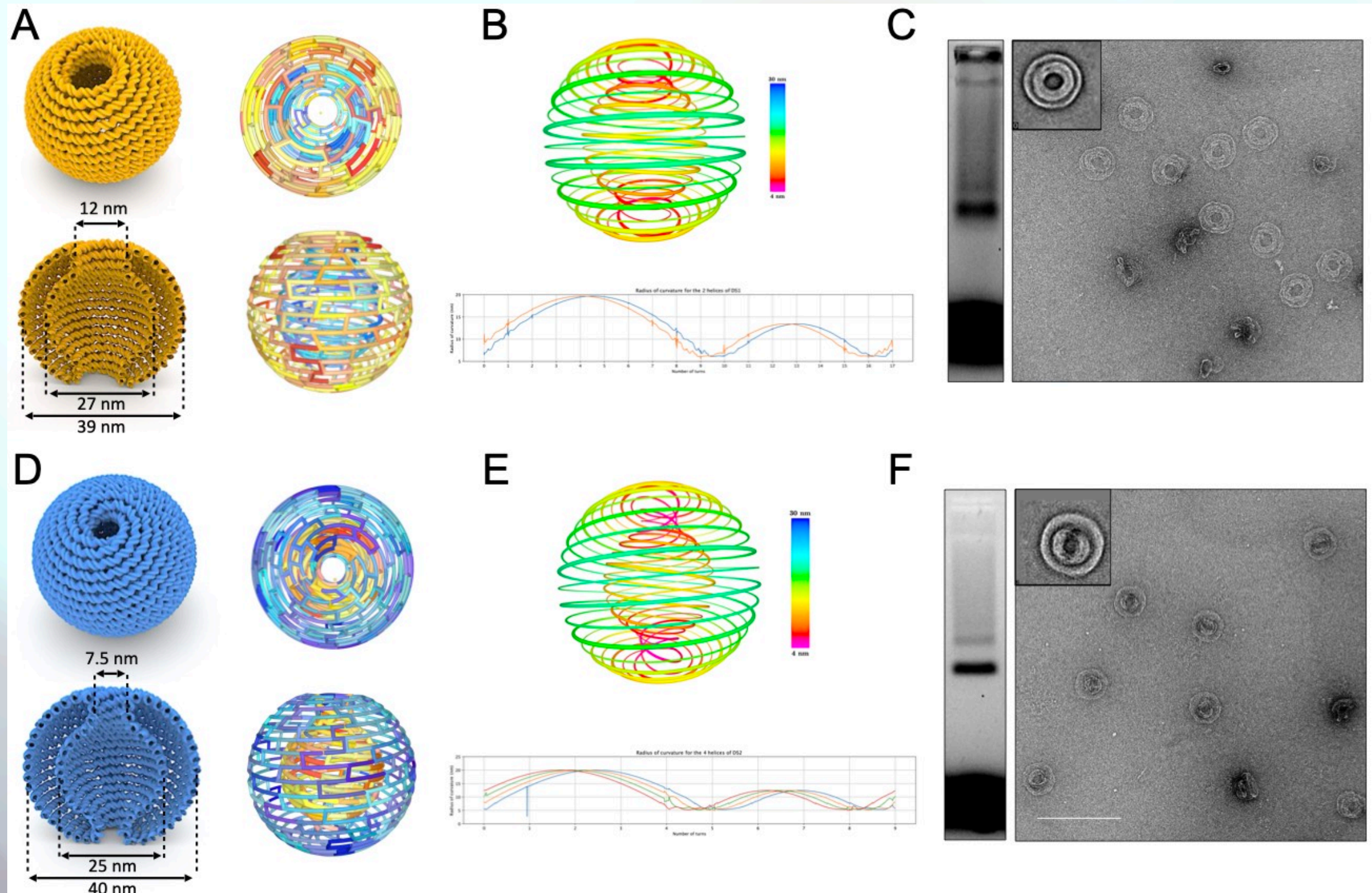
Glimpse of our DNA nanostructures

Experimental work with G. Bellot, J. Finkel and A. Mills



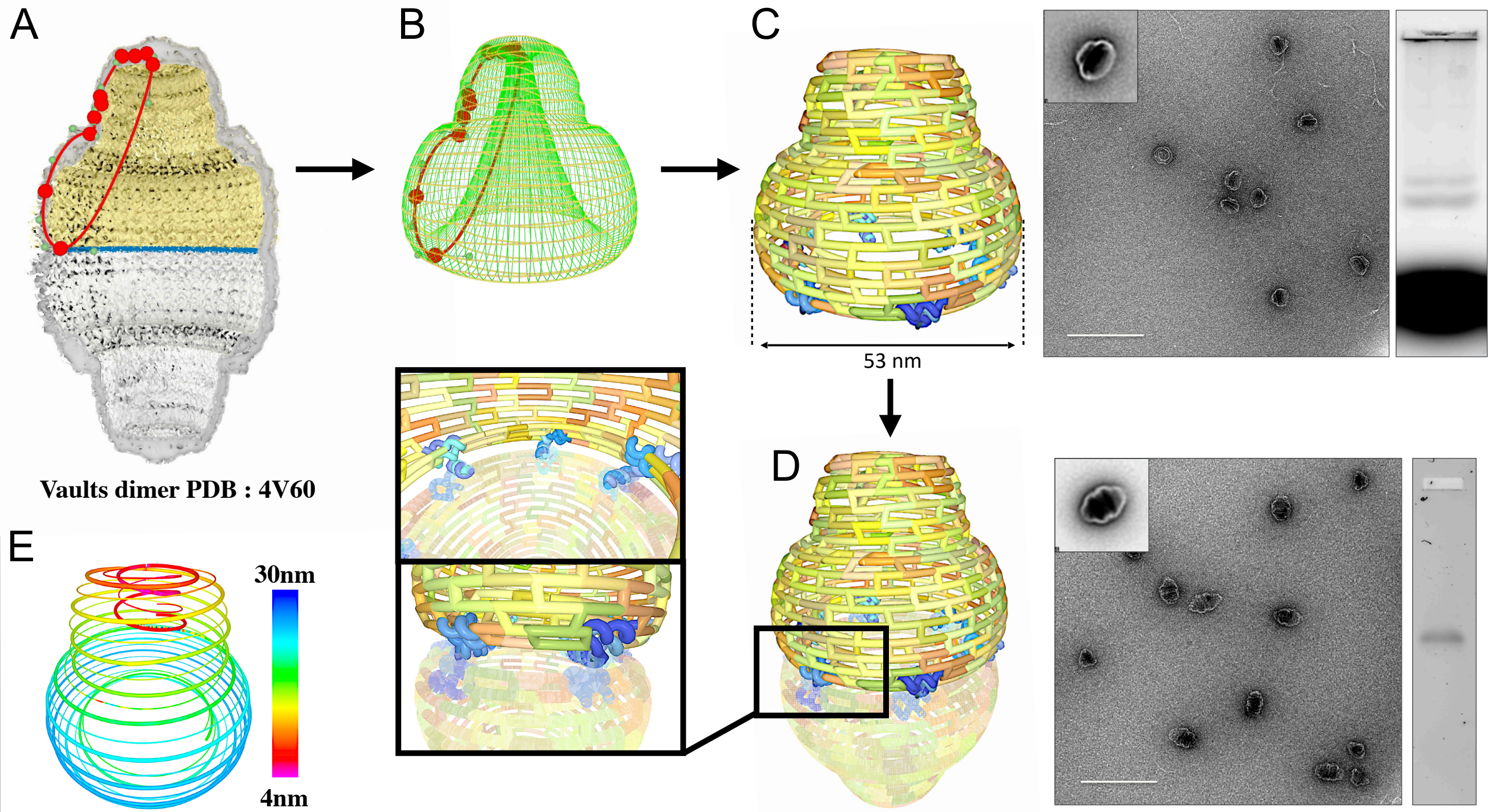
Glimpse of our DNA nanostructures

Experimental work with G. Bellot, J. Finkel and A. Mills



Glimpse of our DNA nanostructures

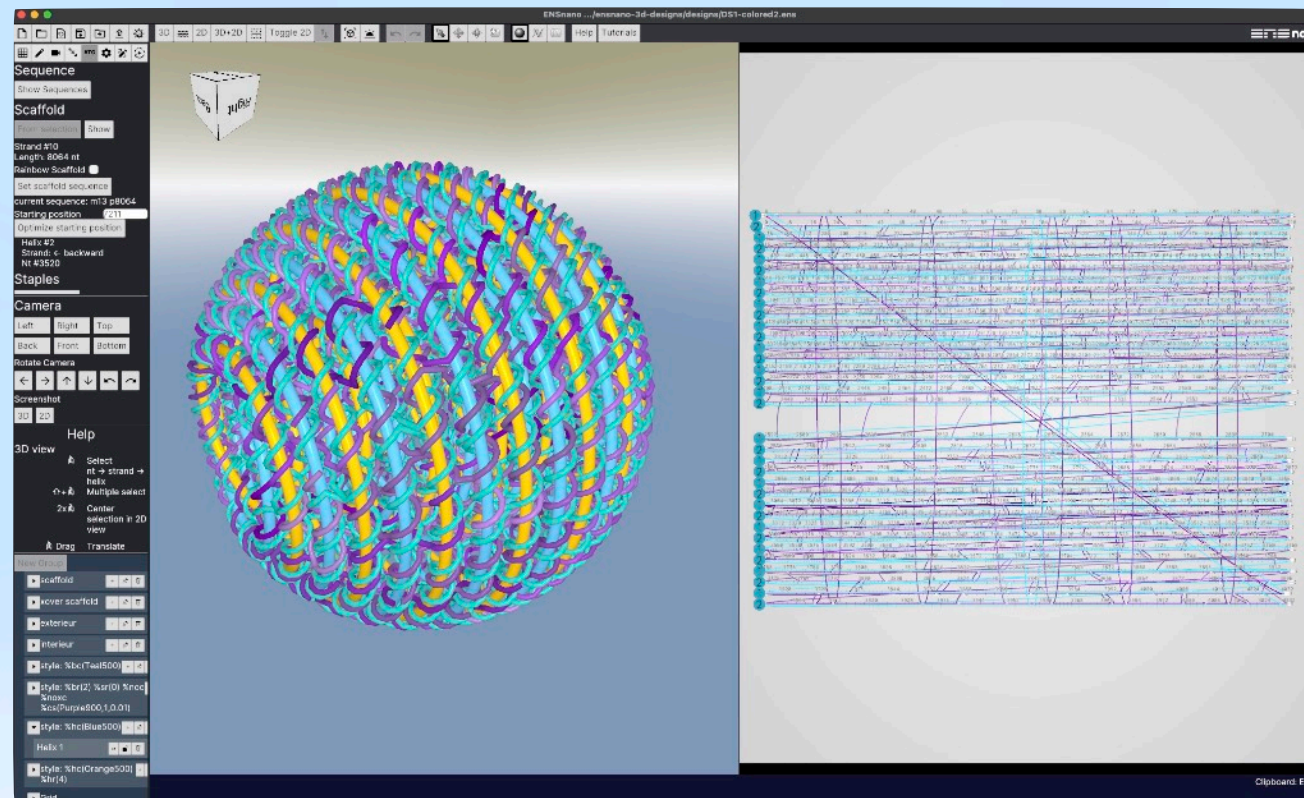
Experimental work with G. Bellot, J. Finkel and A. Mills



**Here is
how we made them**

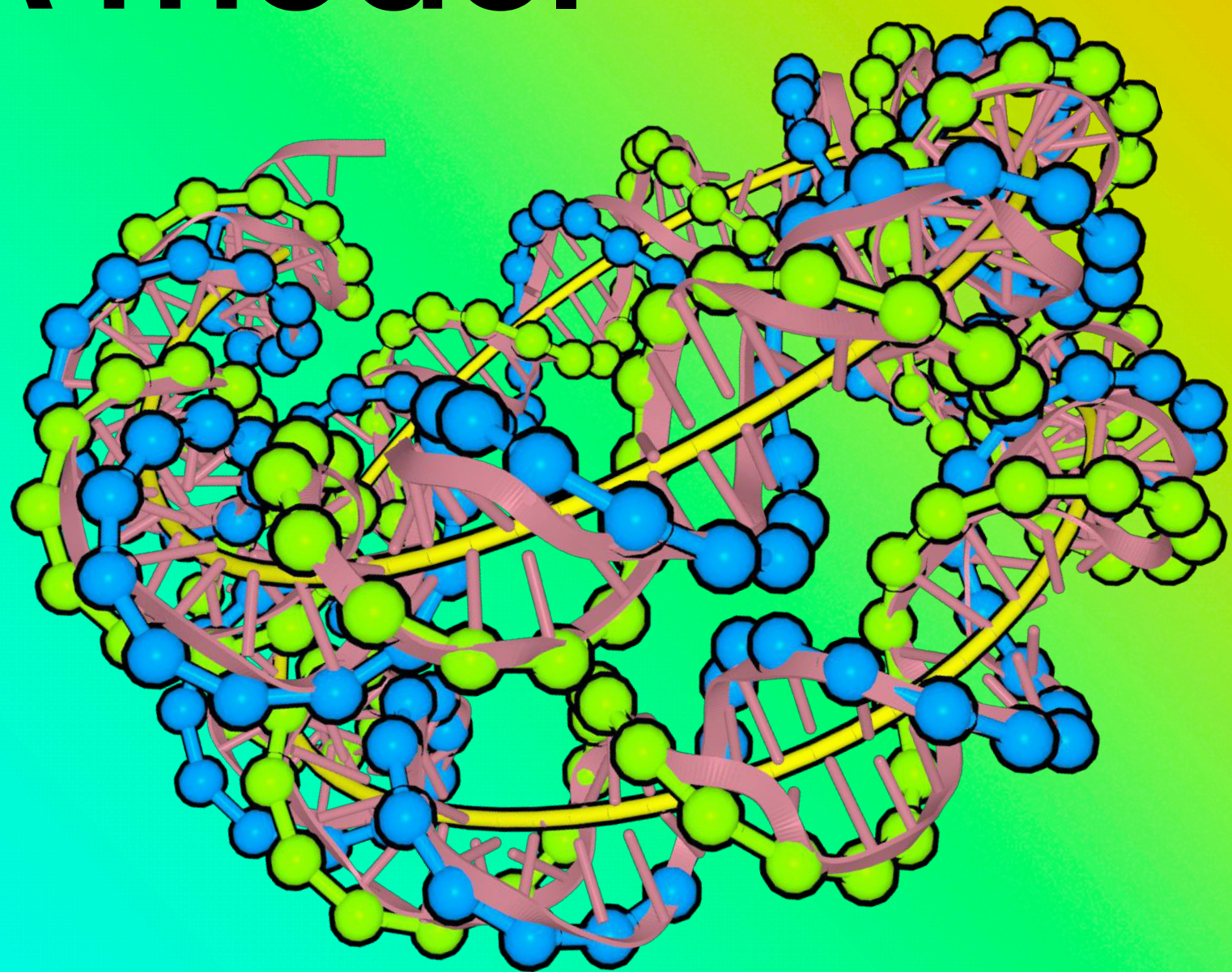


An algorithm & geometry based DNA nanostructure design software



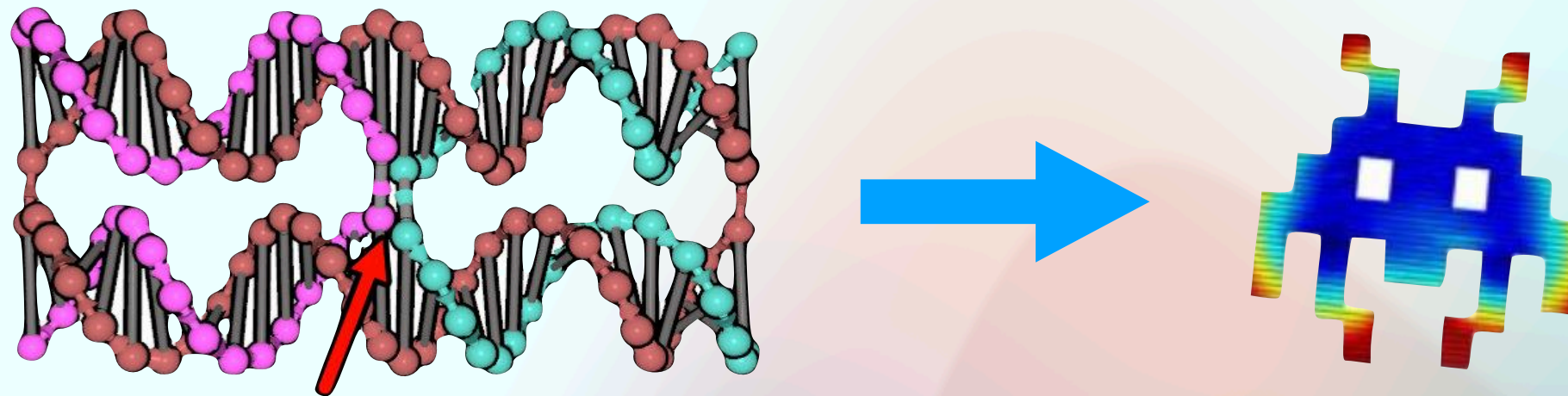
- A dual 3D & 2D interactive interface
- Realtime 3D manipulation
- Easy to use and straightforward to install, multiplatform Windows, Mac OS, Linux
- ~100 000 lines of code, written in Rust 
- Remarquable success in complex 3D DNA nanostructure assembly

○ Defining a good DNA model

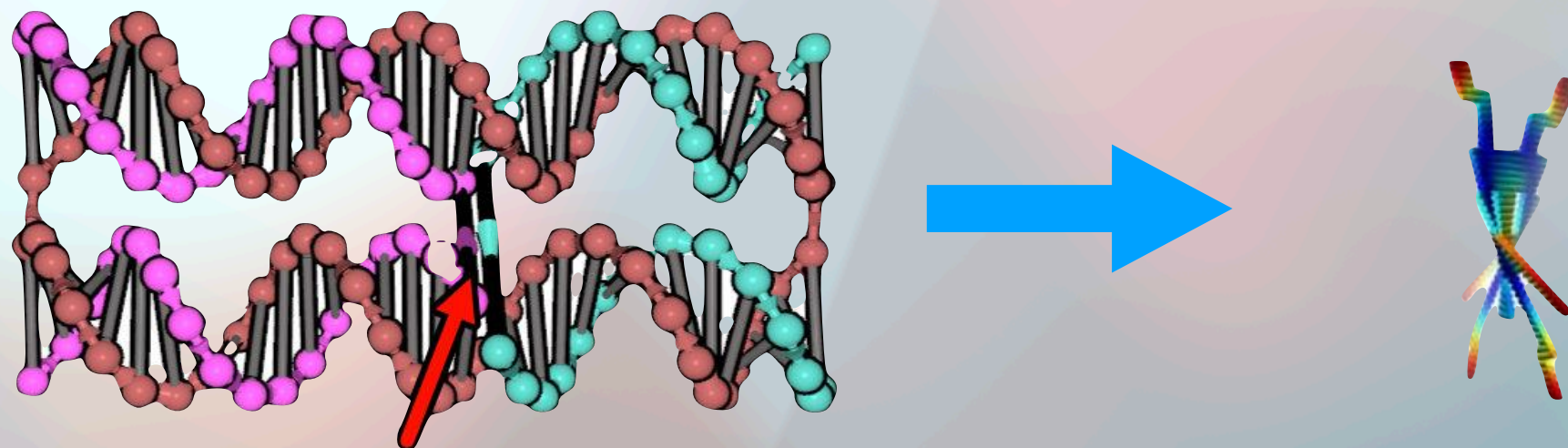


Getting the DNA parameters right

DNA helix parameters matter



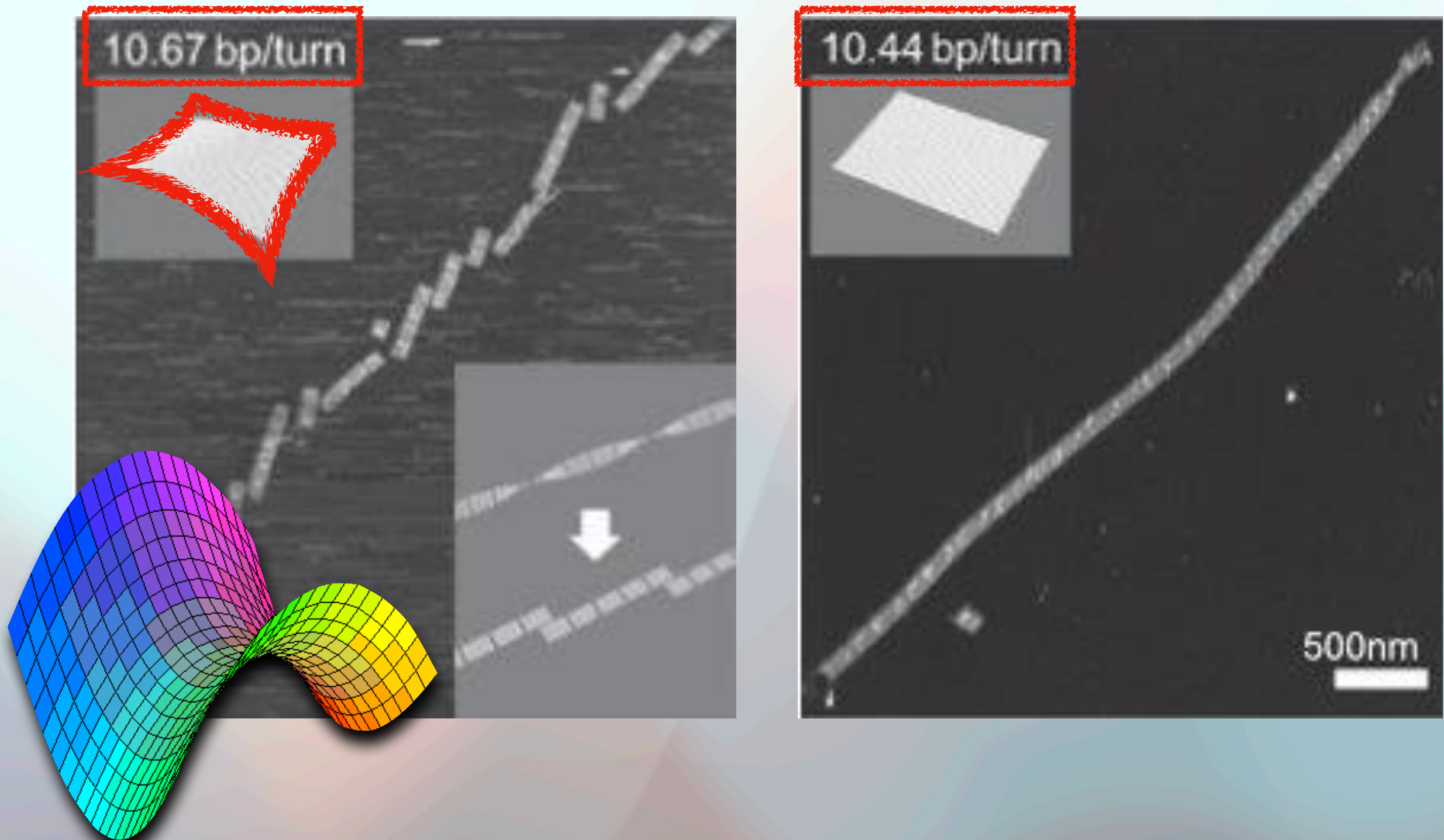
Nucleotides turn by steps of $\sim 34^\circ$ around the helix axis



Shifting a crossover by n positions induces a twist of $\sim n \times 34^\circ$

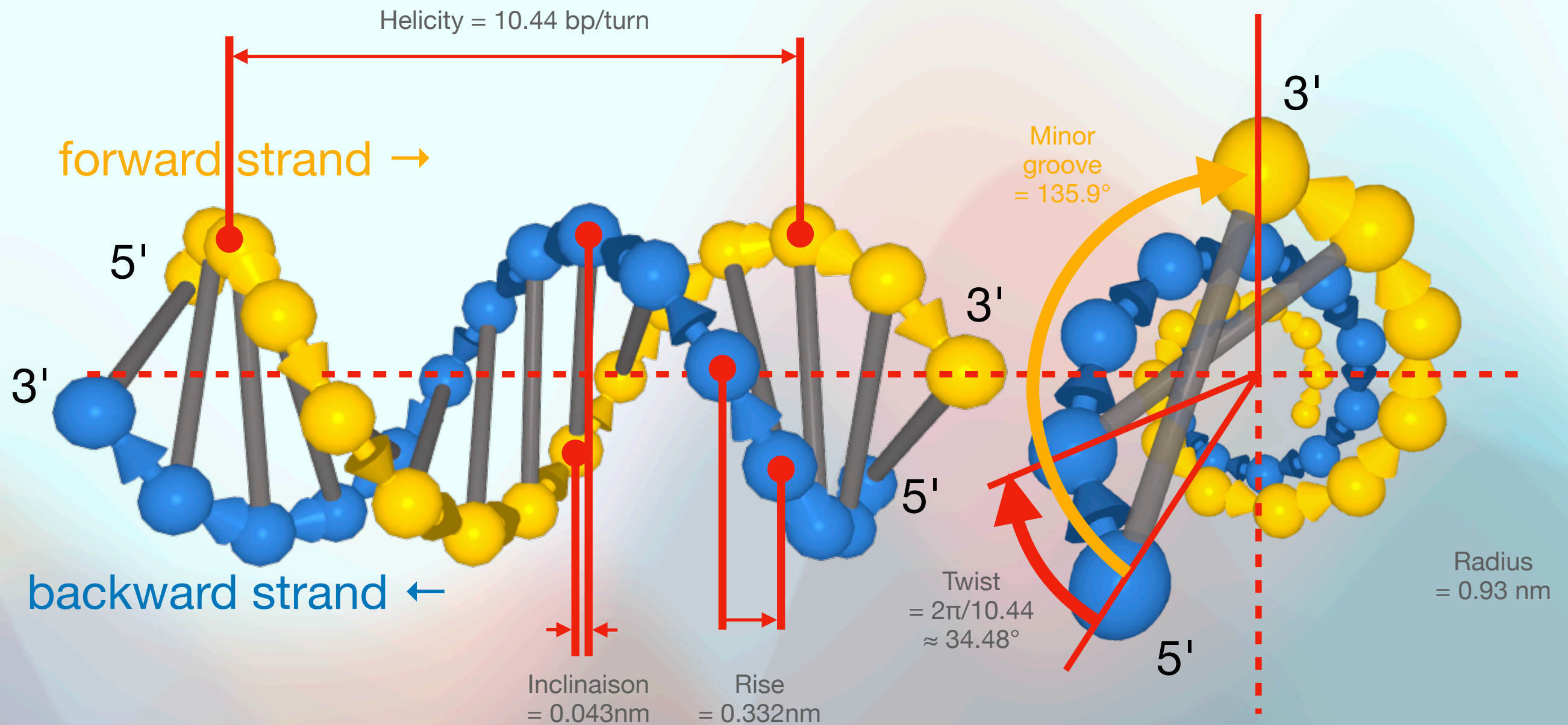
Getting the DNA parameters right

Woo & Rothemund, 2011



Getting the DNA parameters right

Good parameters for straight DNA double helices (B-form)

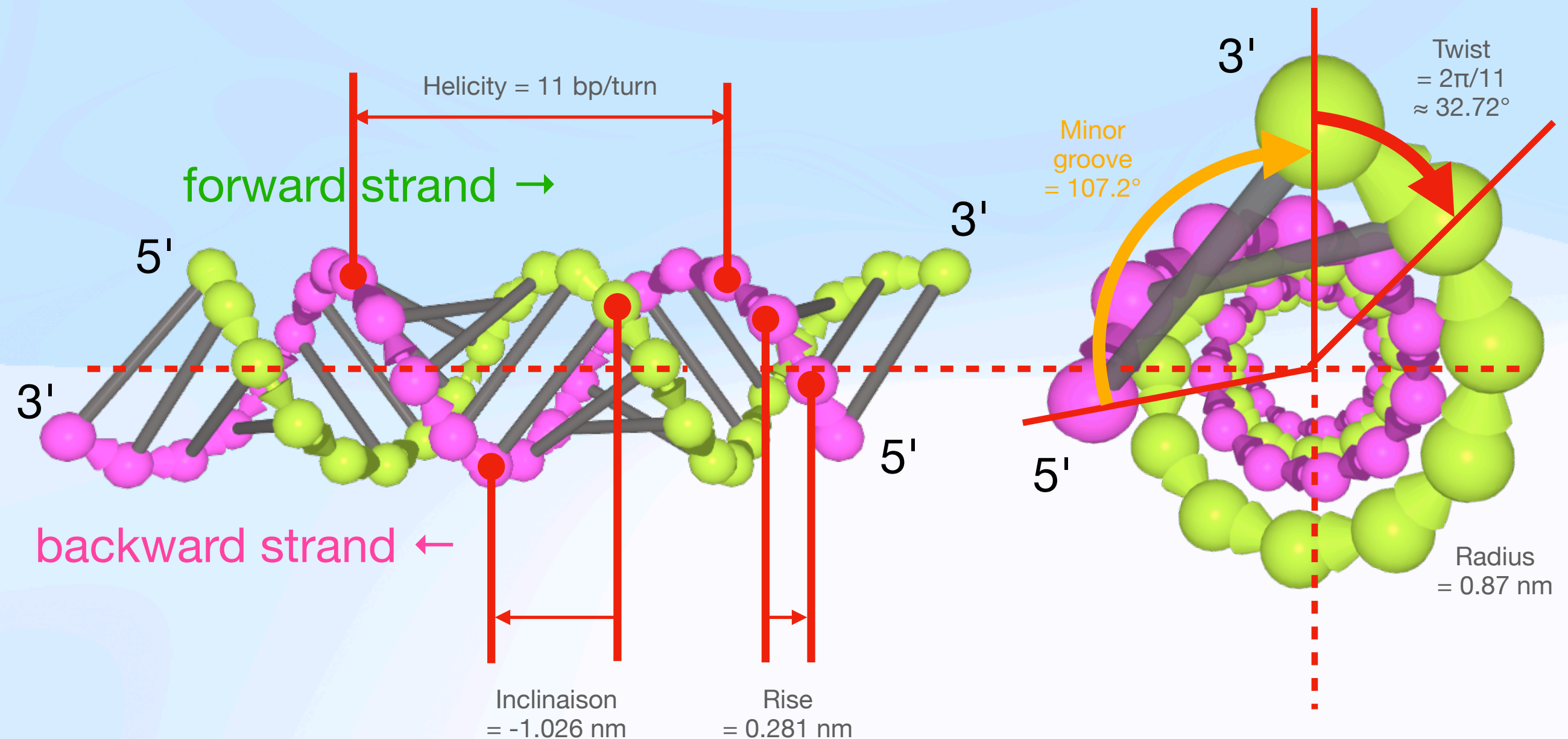


Inter helix axis distance: 2.65 nm

Woo, Rothmund, 2011; Cody, Andersen, 2014;
Thubagere *et al* 2017

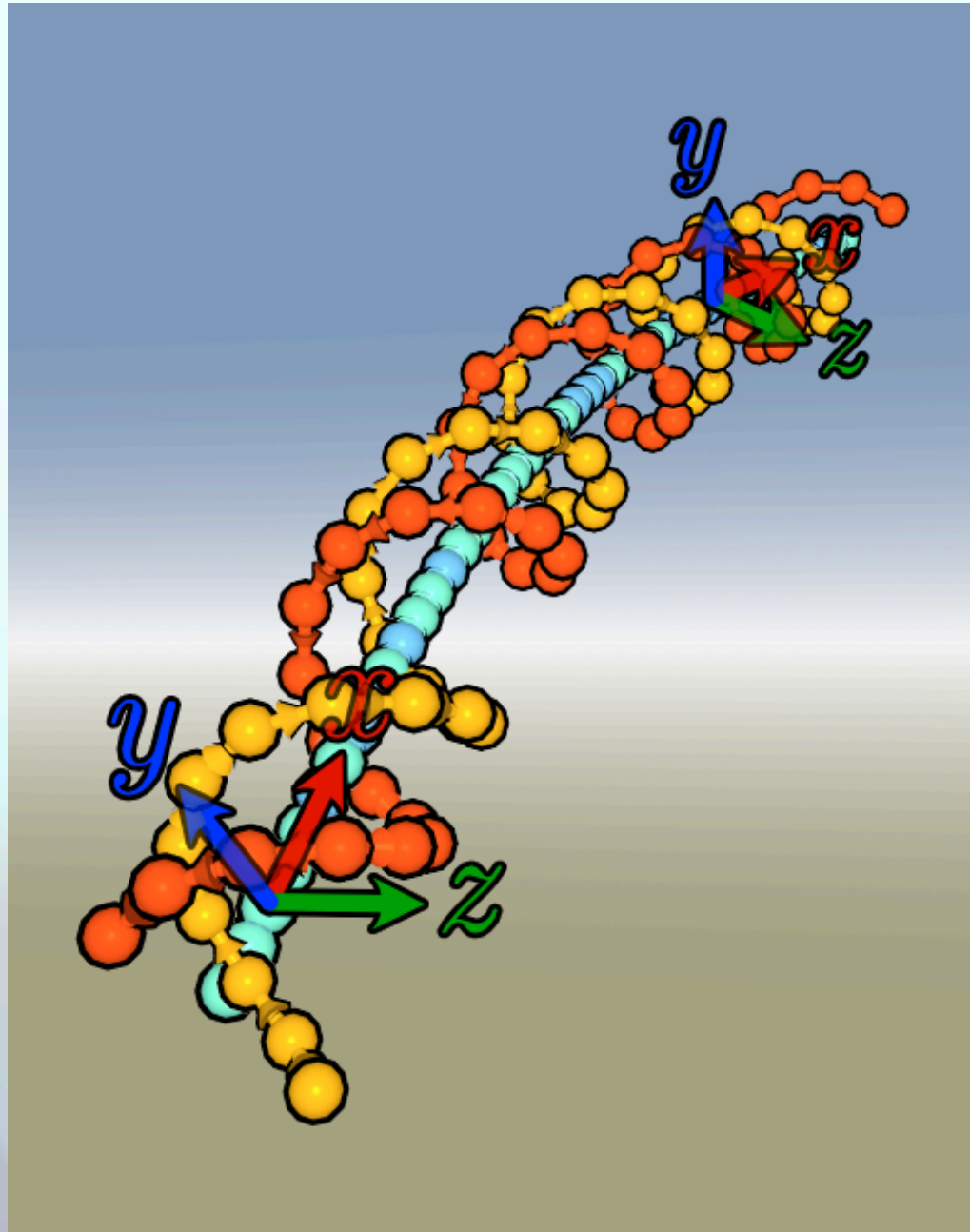
Getting the RNA parameters right

Good parameters for straight RNA double helices (A-form)



Mapping DNA helix on a curve

Finding a proper frame that runs along the curve



What about the Frenet frame?

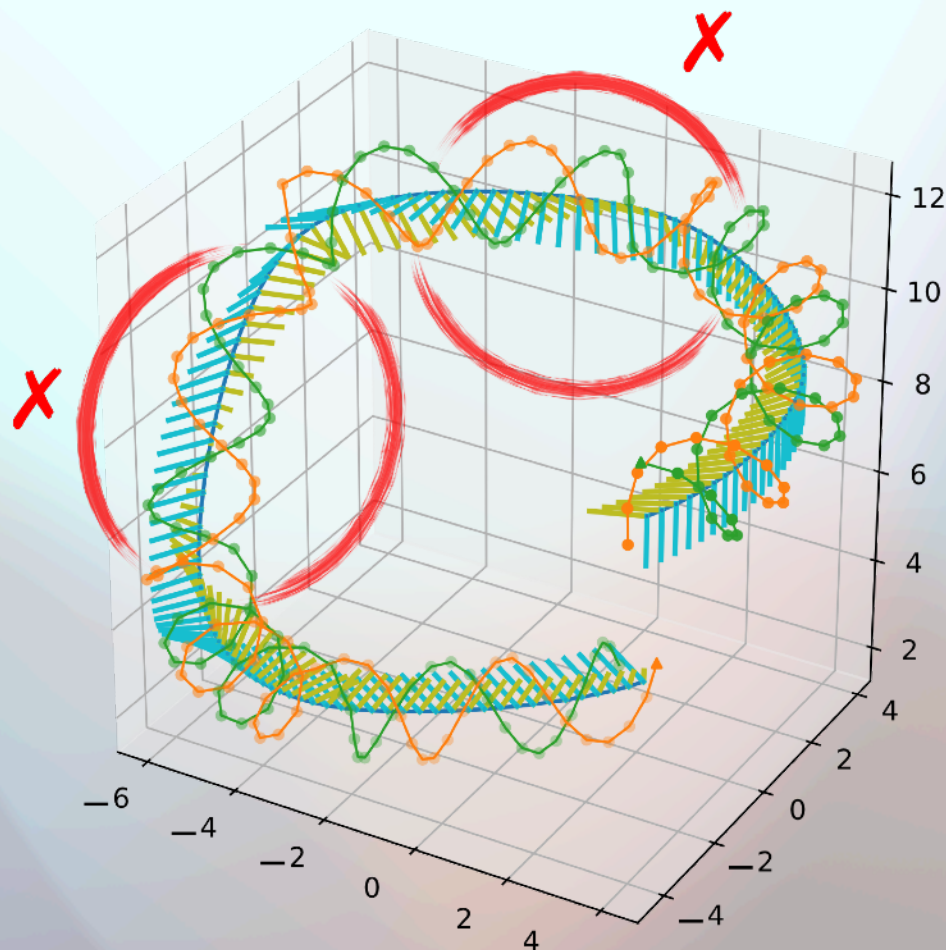
- Reparametrize $M(t)$ by the curvilinear abscissa: $s(t) = \int_0^t ||\dot{M}(t)|| dt$
- $x(s) = dM(s) / ds$, the *tangent*
- $y(s) = \text{Normalized}(d^2M(s) / d^2s)$, the *normal*
- $z(s) = x(s) \wedge y(s)$

Mapping DNA helix on a curve

Finding a proper frame that runs along the curve

**Frenet frame
flips
"for no reason"**

Frenet frame algorithm

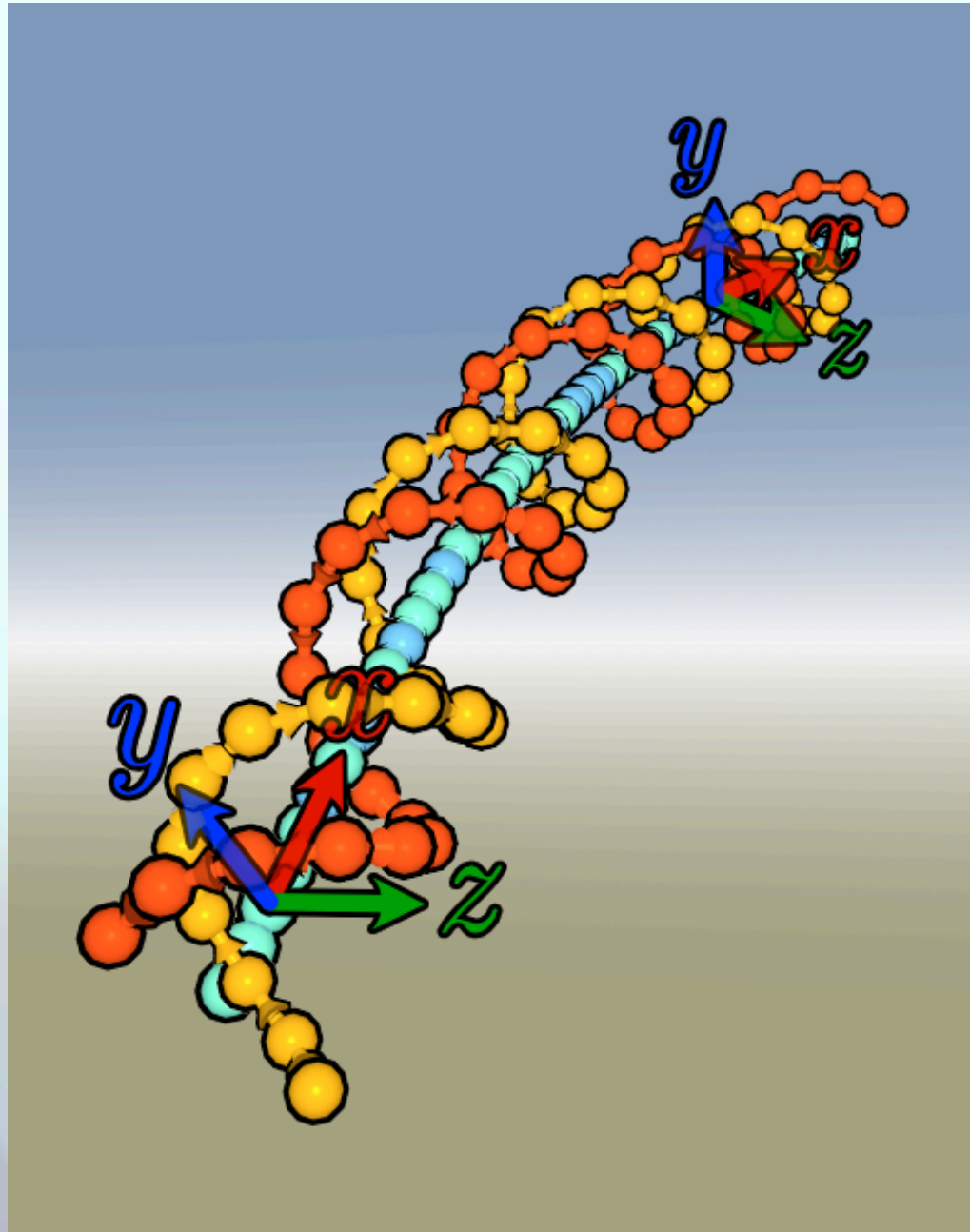


What about the Frenet frame?

- Reparametrize $M(t)$ by the curvilinear abscissa: $s(t) = \int_0^t ||\dot{M}(t)|| dt$
- $x(s) = dM(s) / ds$, the *tangent*
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- $z(s) = x(s) \wedge y(s)$

Mapping DNA helix on a curve

Finding a proper frame that runs along the curve



We propose an iterative frame:

- Let $t_1 \leq \dots \leq t_k$ the positions of the points
- $X_k = \overrightarrow{T}(t_k)$, the *tangent* at $M(t_k)$
- $Y_k = \text{rot}_{X_{k-1} \rightarrow X_k}(Y_{k-1})$
- $Z_k = \text{rot}_{X_{k-1} \rightarrow X_k}(Z_{k-1})$

where $\text{rot}_{X_{k-1} \rightarrow X_k}$ denotes the rotation from X_{k-1} to X_k around $X_{k-1} \wedge X_k$

Mapping DNA helix on a curve

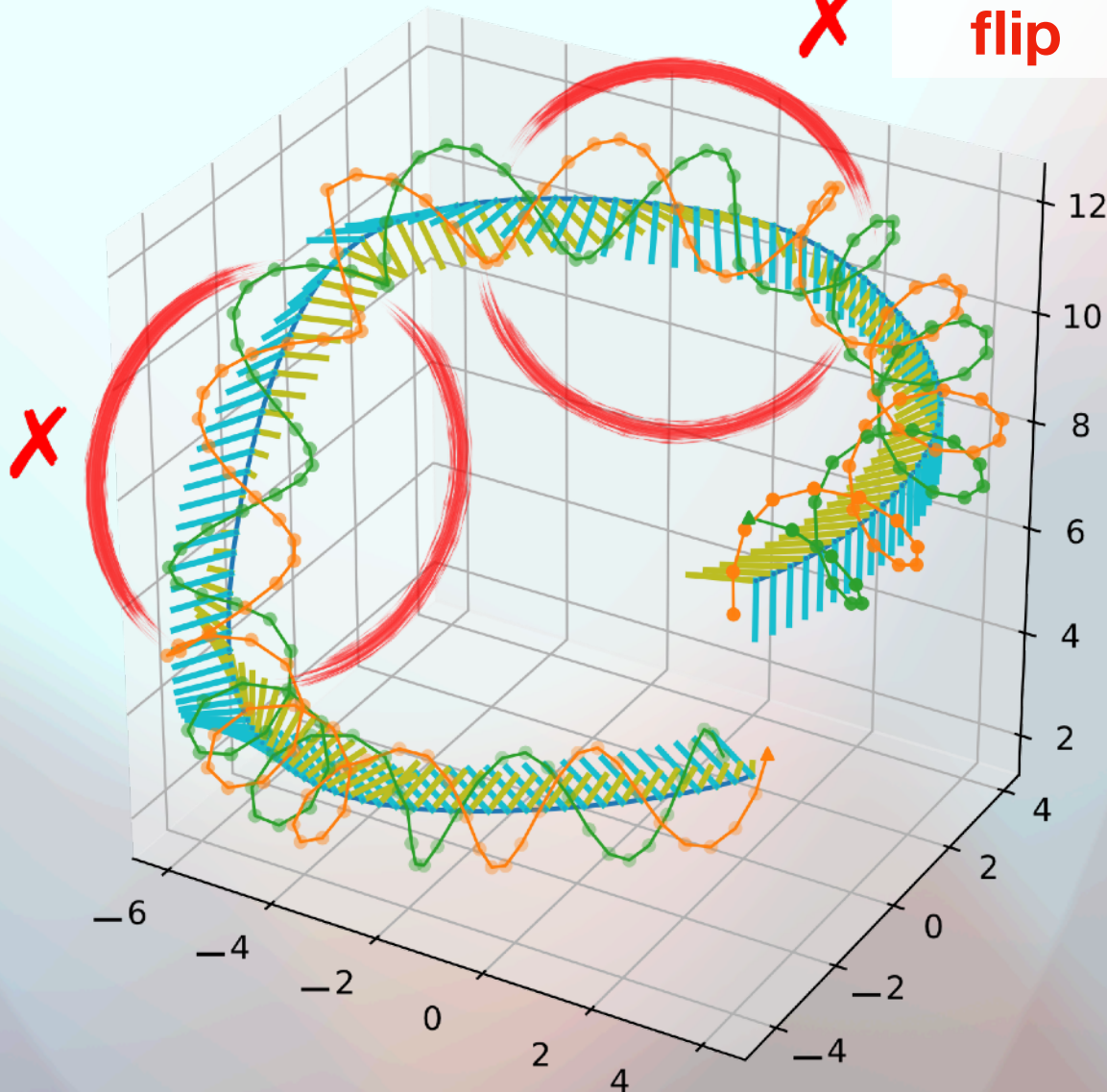
Frenet frame vs our iterative frame

Frenet frame

Frenet frame algorithm

✗

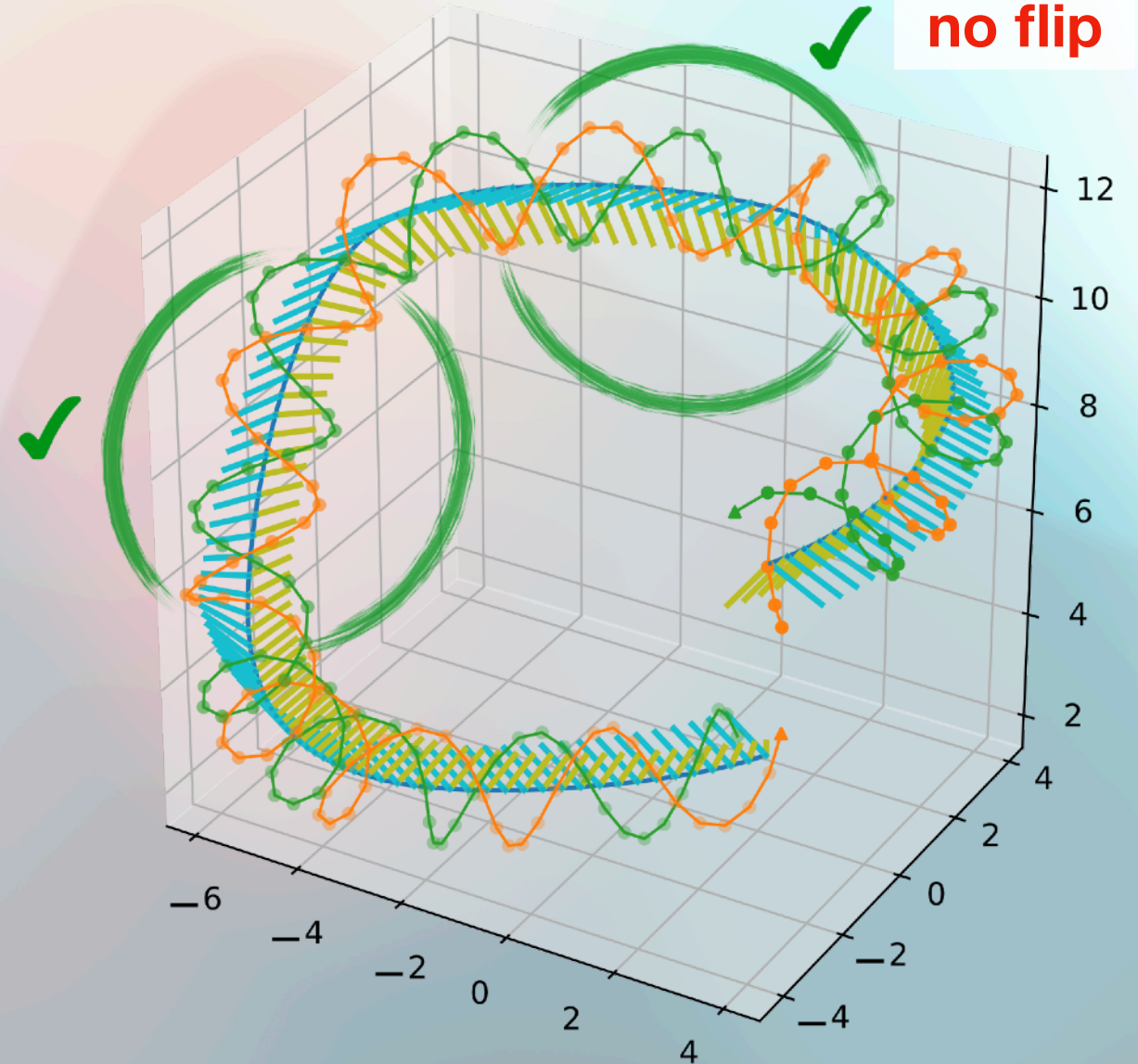
flip



Our iterative frame

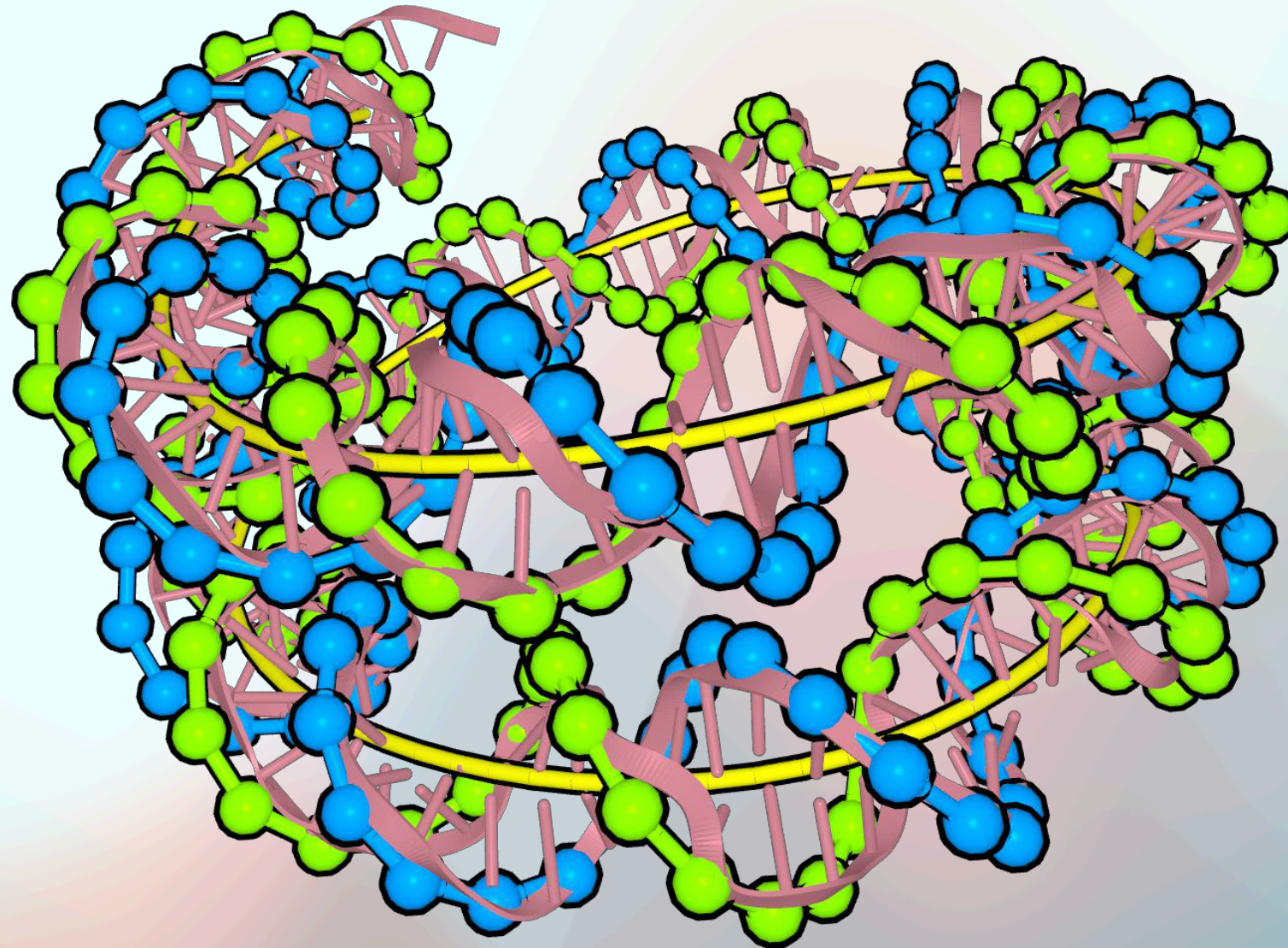
Iterative frame algorithm

✓ no flip



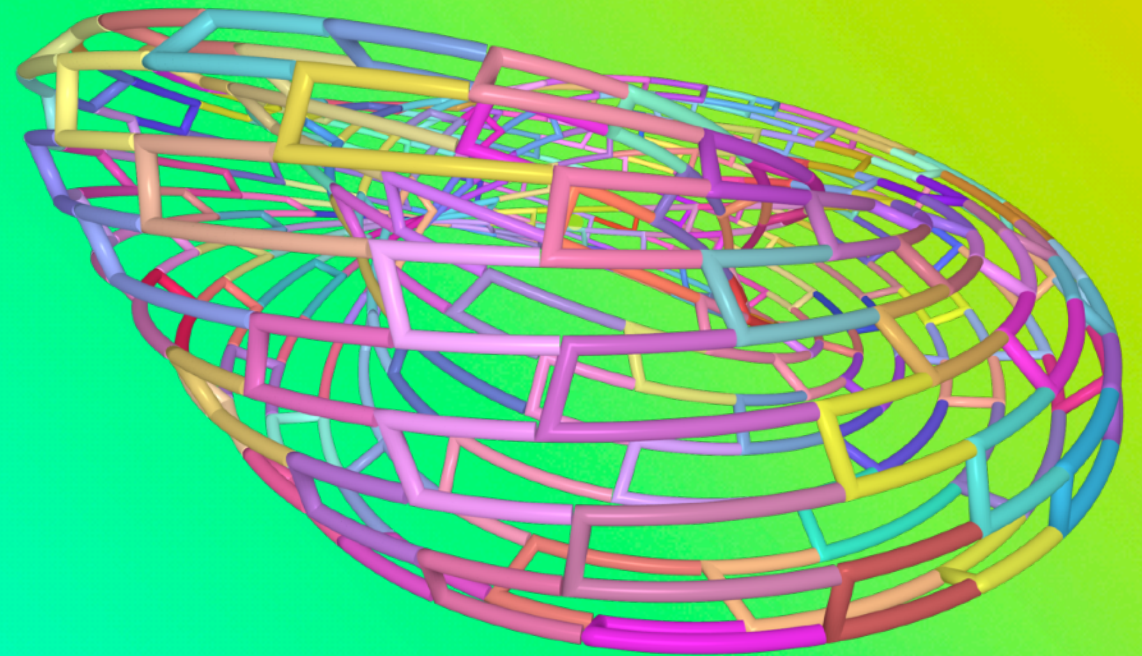
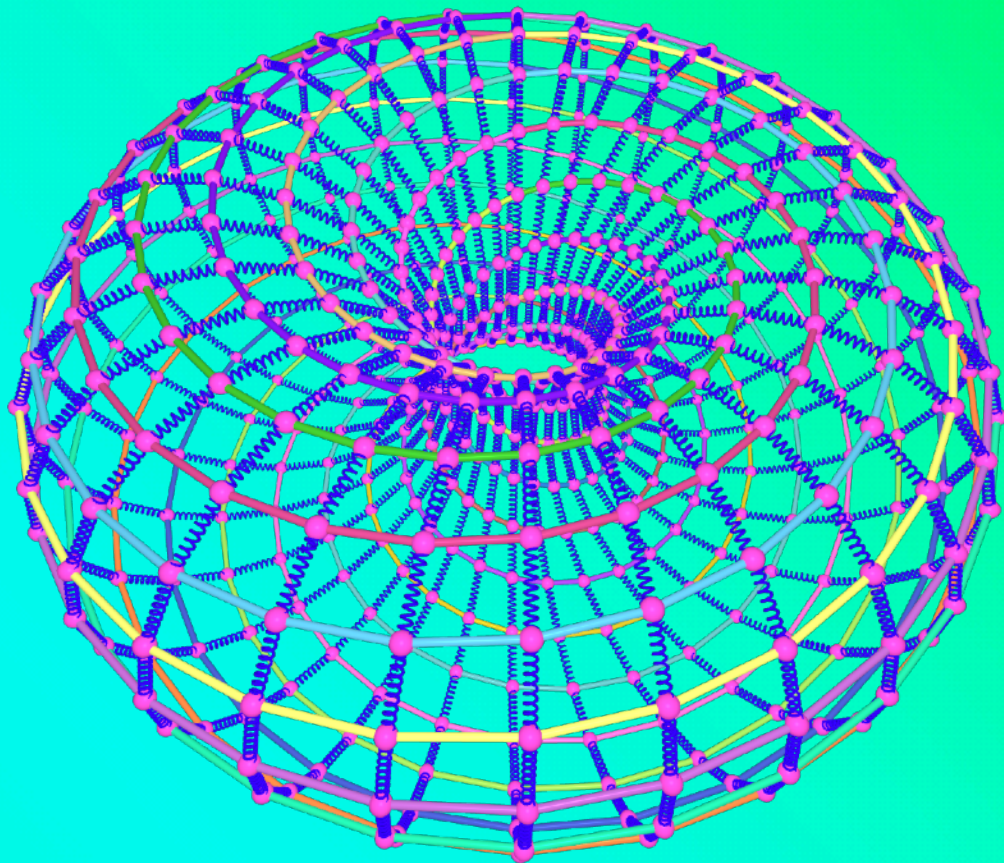
Mapping DNA helix on a curve

Our model vs crystallographic data



A nucleosome (curvature radius $\sim 6\text{nm}$)

Making a DNA origami

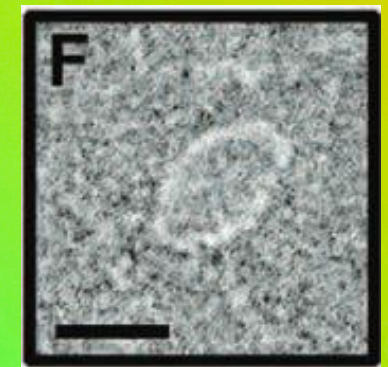
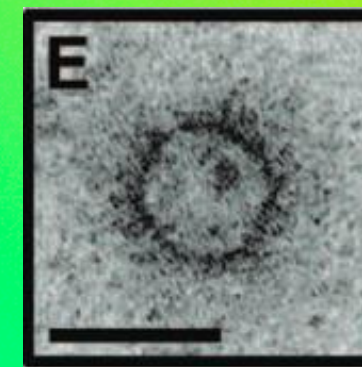
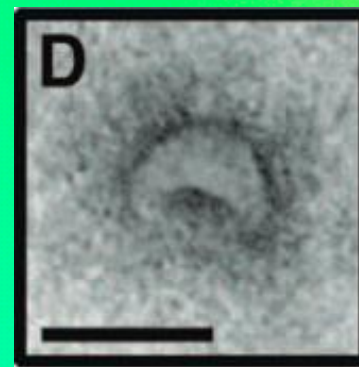
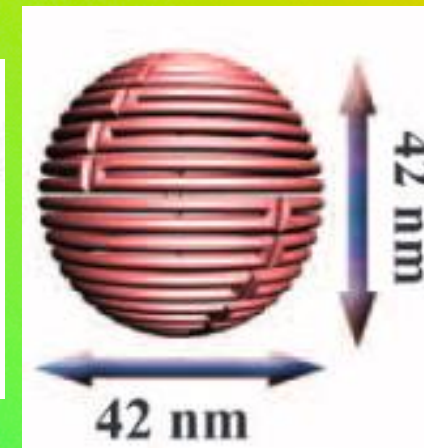
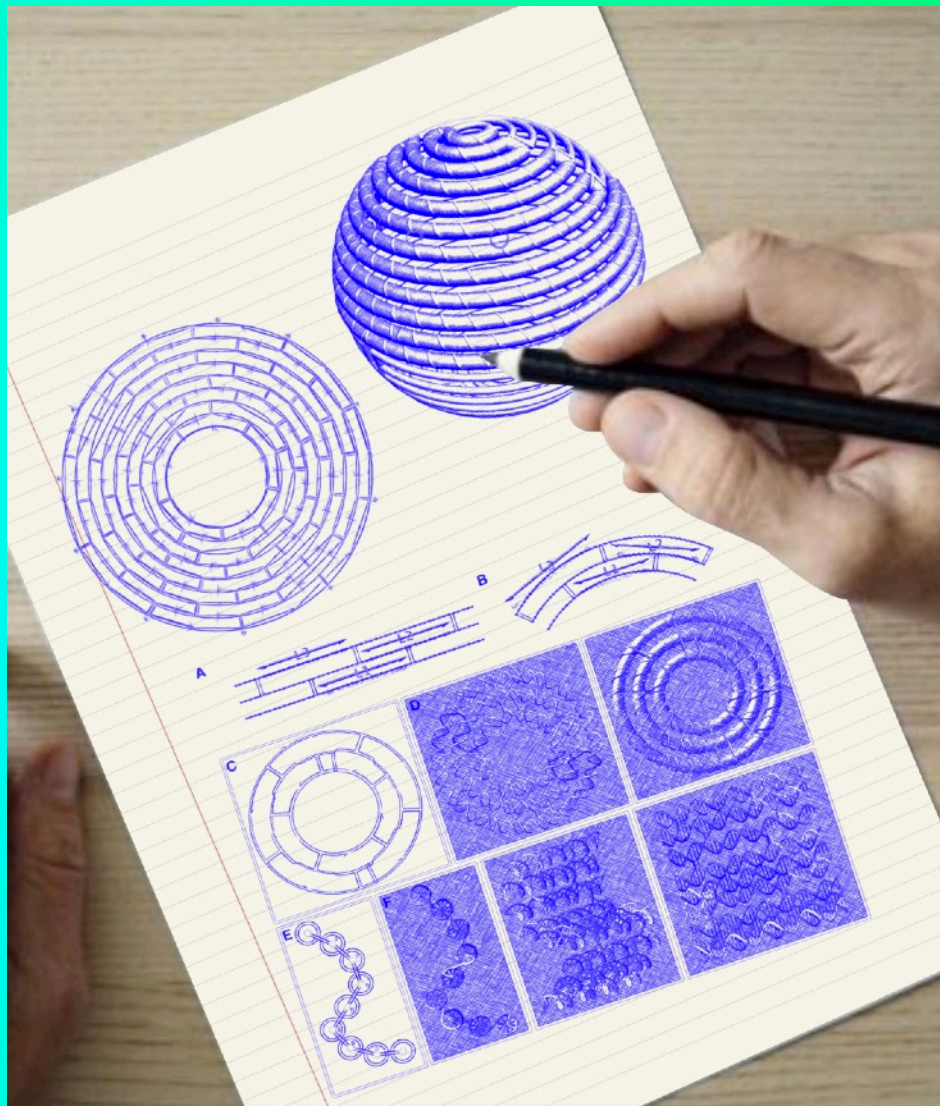


Current recipe for designing a DNA origami

The main steps of the process

1. **Scaling** the object and **placing the DNA helices**
2. **Optimizing the DNA parameters** for each helix to spread the strain
3. **Routing the scaffold** strand to cover all the helices
4. **Stapling** the design to tie it together

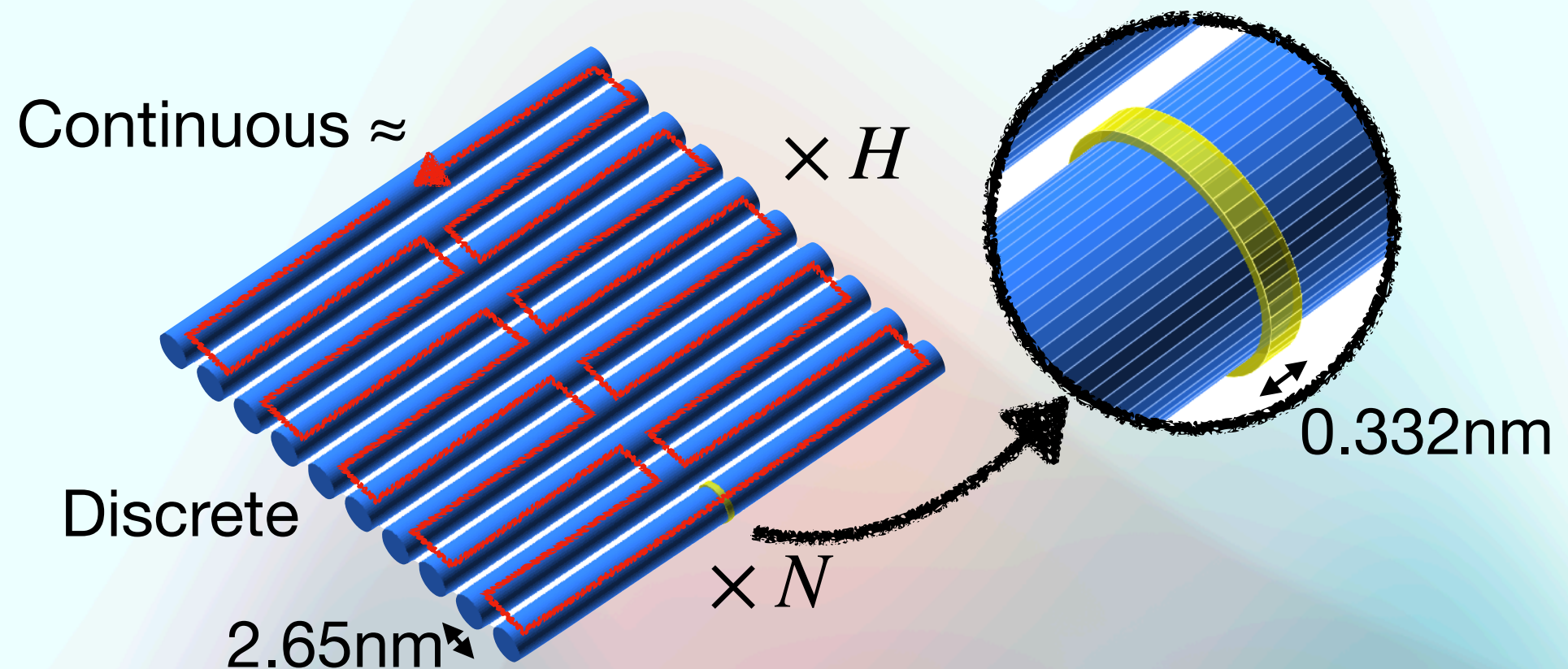
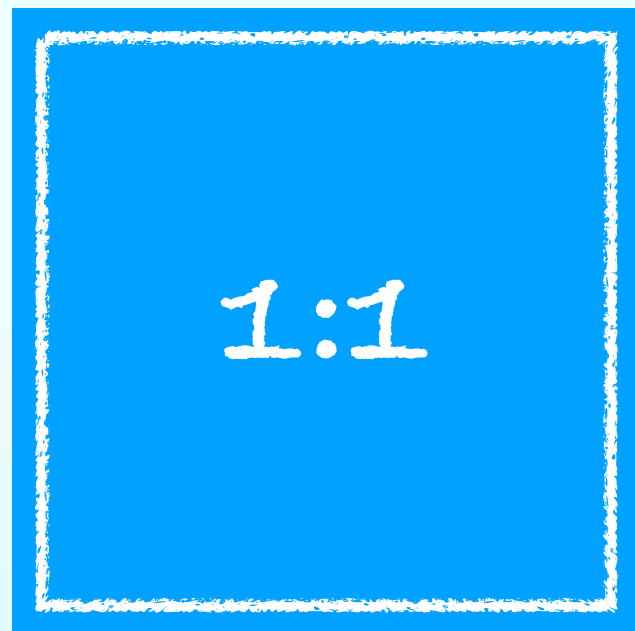
1. Looking for the right scale



“At this point, **manual consideration of the complex relations among the design parameters is the most time-consuming step** and requires a substantial understanding of fundamental engineering principles and the behavior of interconnected DNA.” Han et al., 2011

The case of planar designs

semi-discrete & semi-continuous

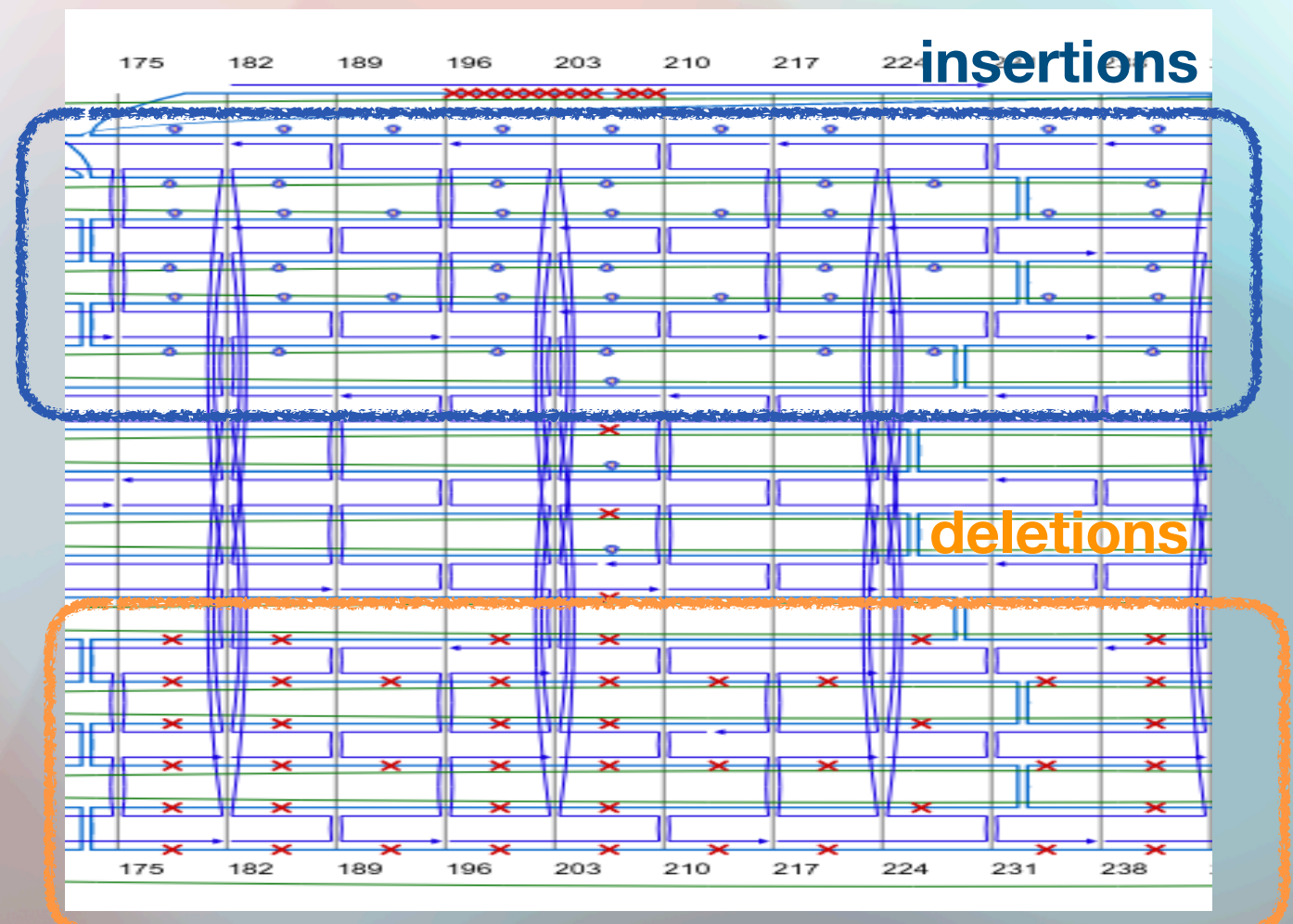
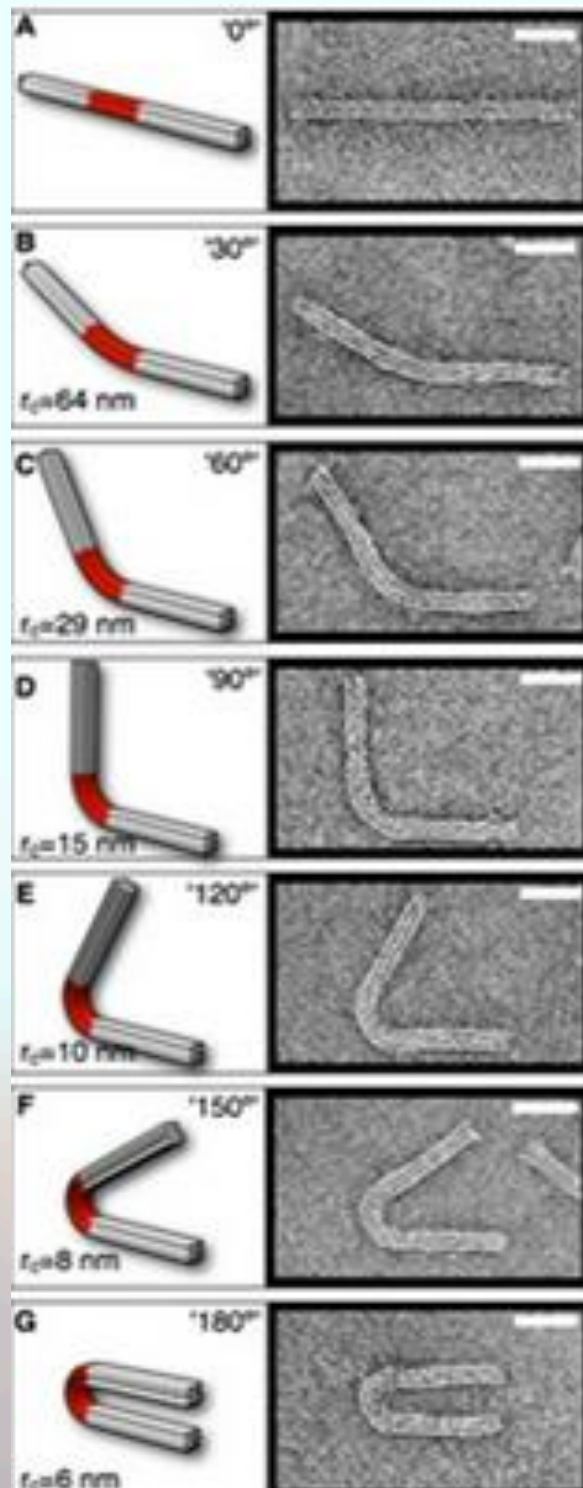


Solve

- Aspect ratio: $2 + (H - 1) \times 2.65 = N \times 0.332$
- Scaffold length: $N \times H \leq 7249$ base pairs
- Scaffold routing: $N = 10.44 \times T$ for T integral number of turns

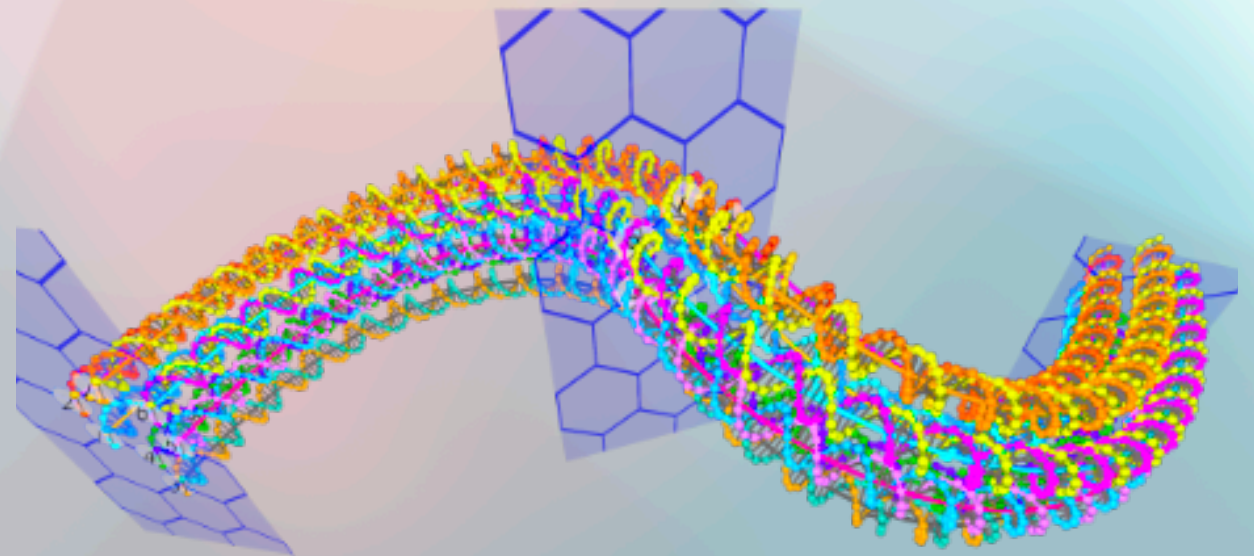
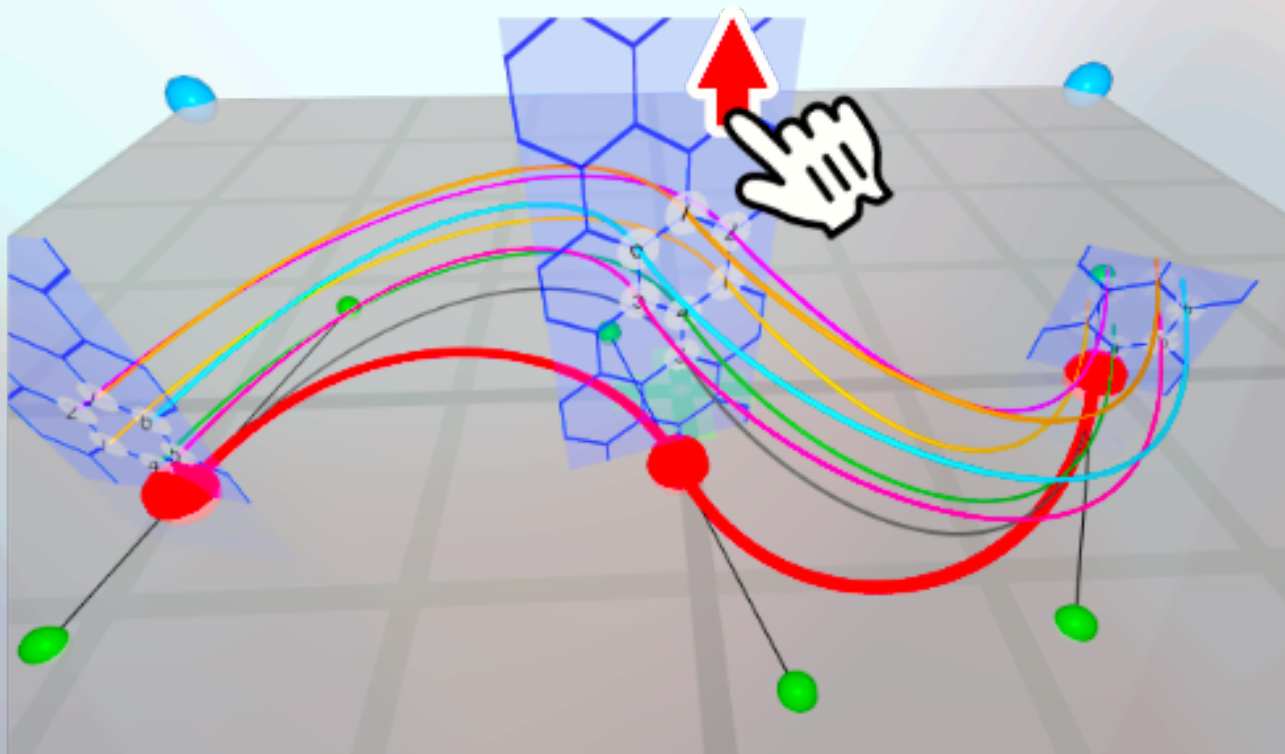
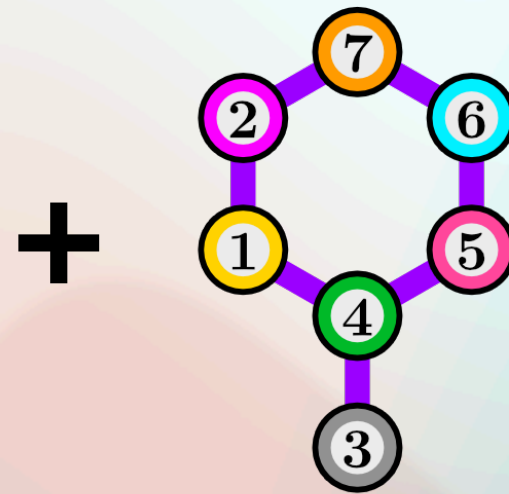
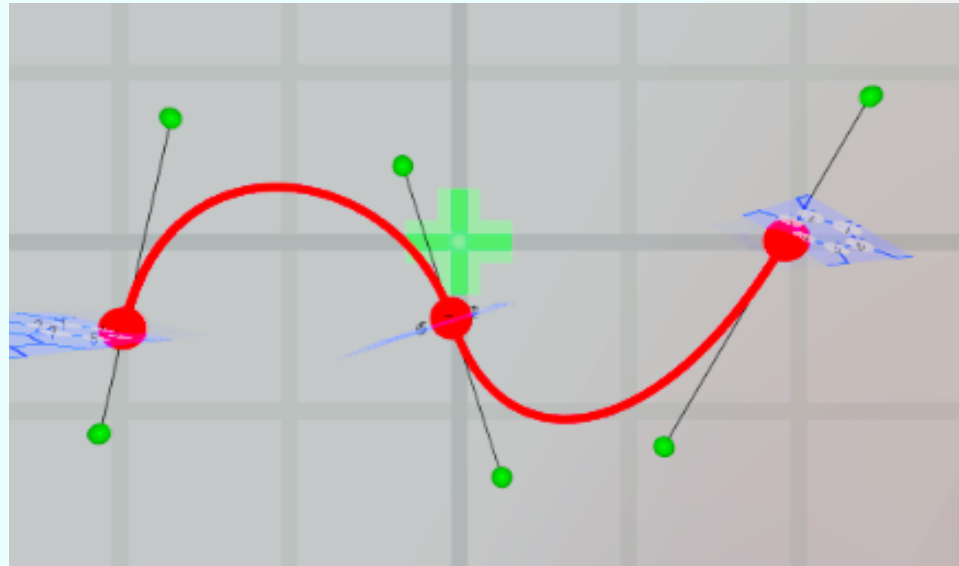
Curved origami with Cadnano

Insertions & deletions map



The case of curved bundle

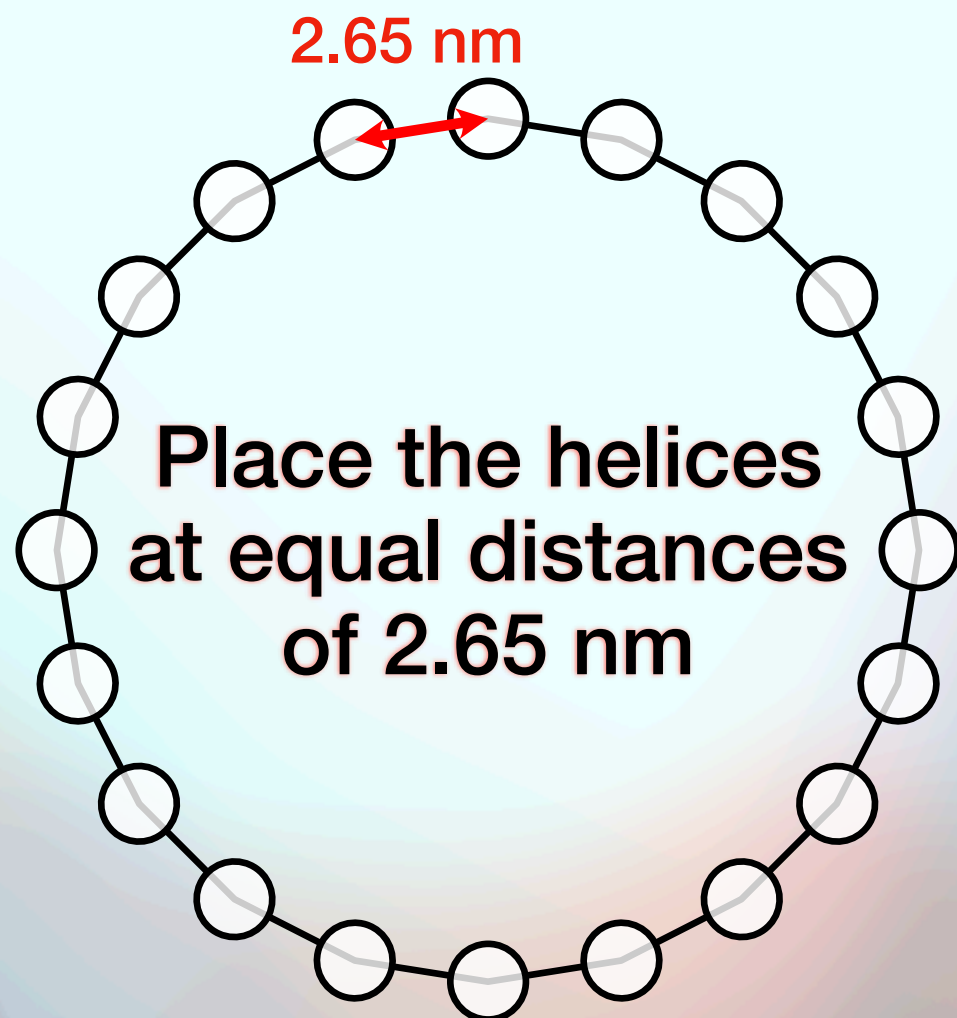
Bezier based in  nano



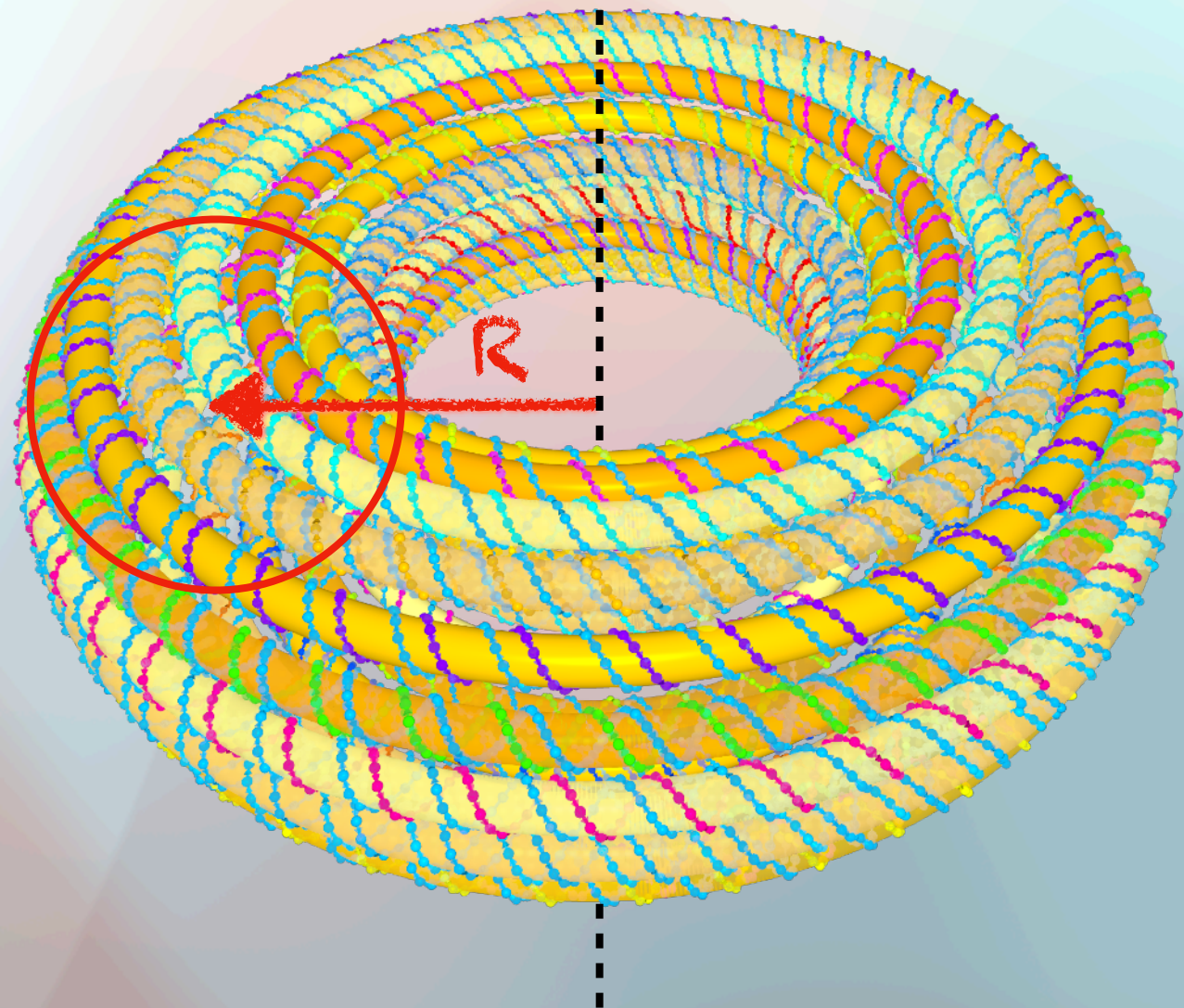
Scale the curve so that the total length of the curves equals the scaffold length

The case of curved shapes

Using concentric circles

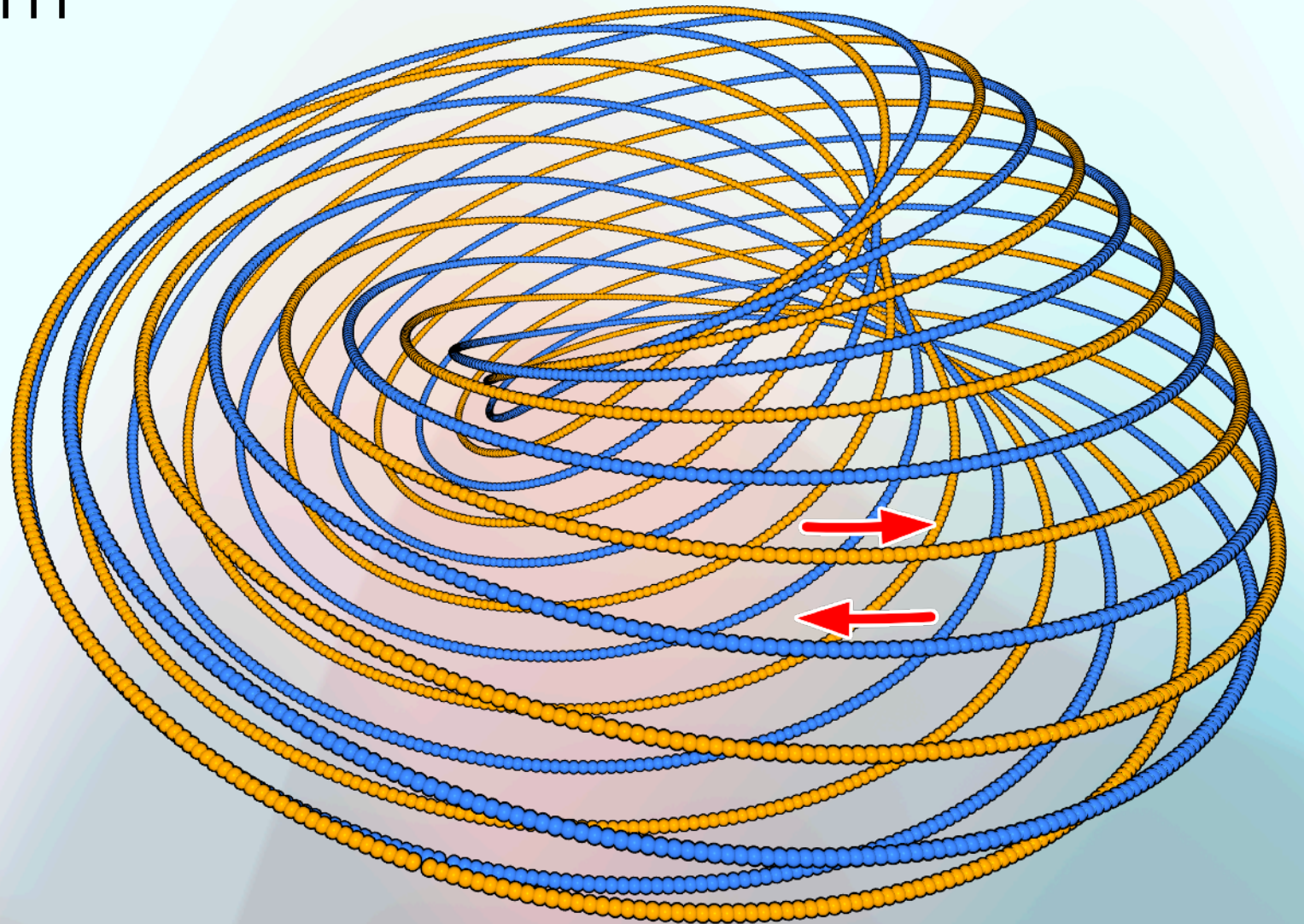


Scale the **revolution radius R** until the total length equals the scaffold length



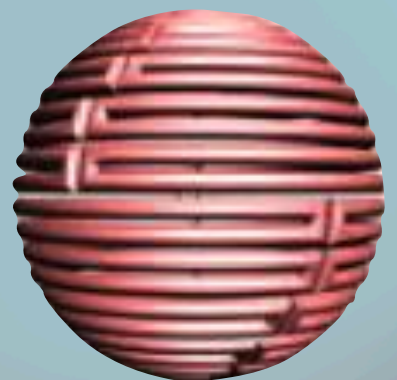
The case of tight curved shapes

A new spiral paradigm



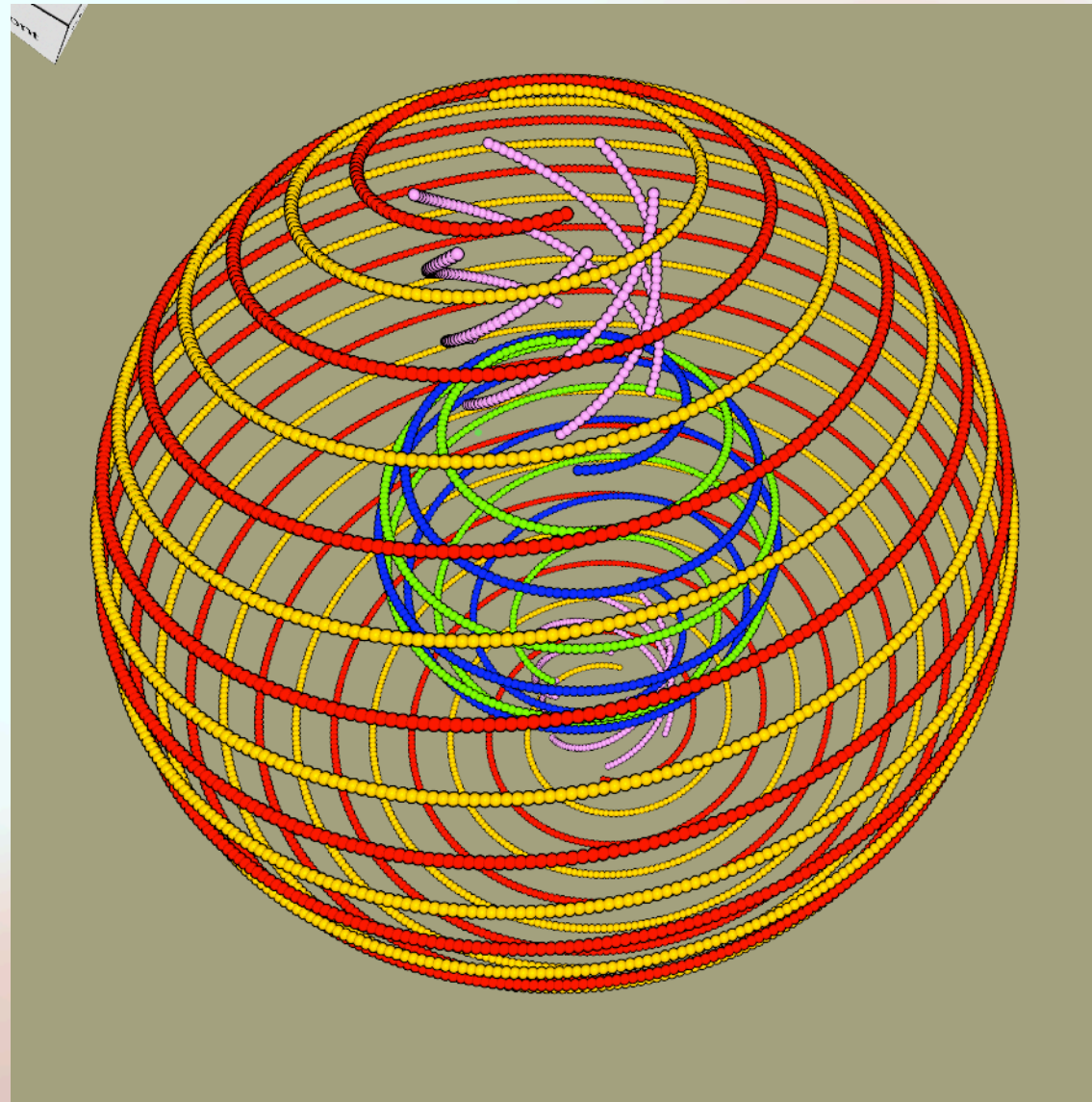
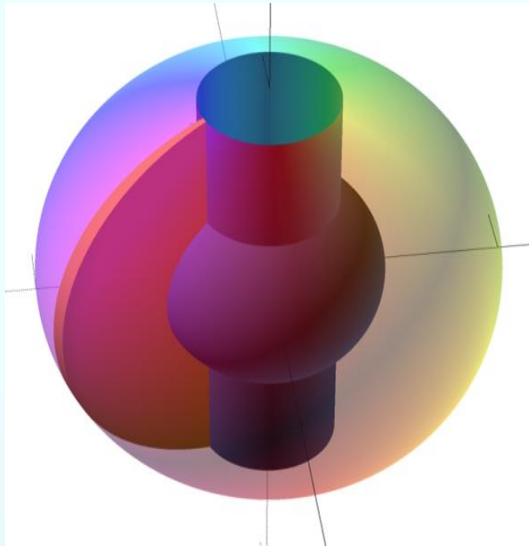
Advantages:

- **Continuous range** of angles between neighboring helices
- Very long helices **reduces considerably the impact of discretization** (~3600 nts long instead of few hundreds)



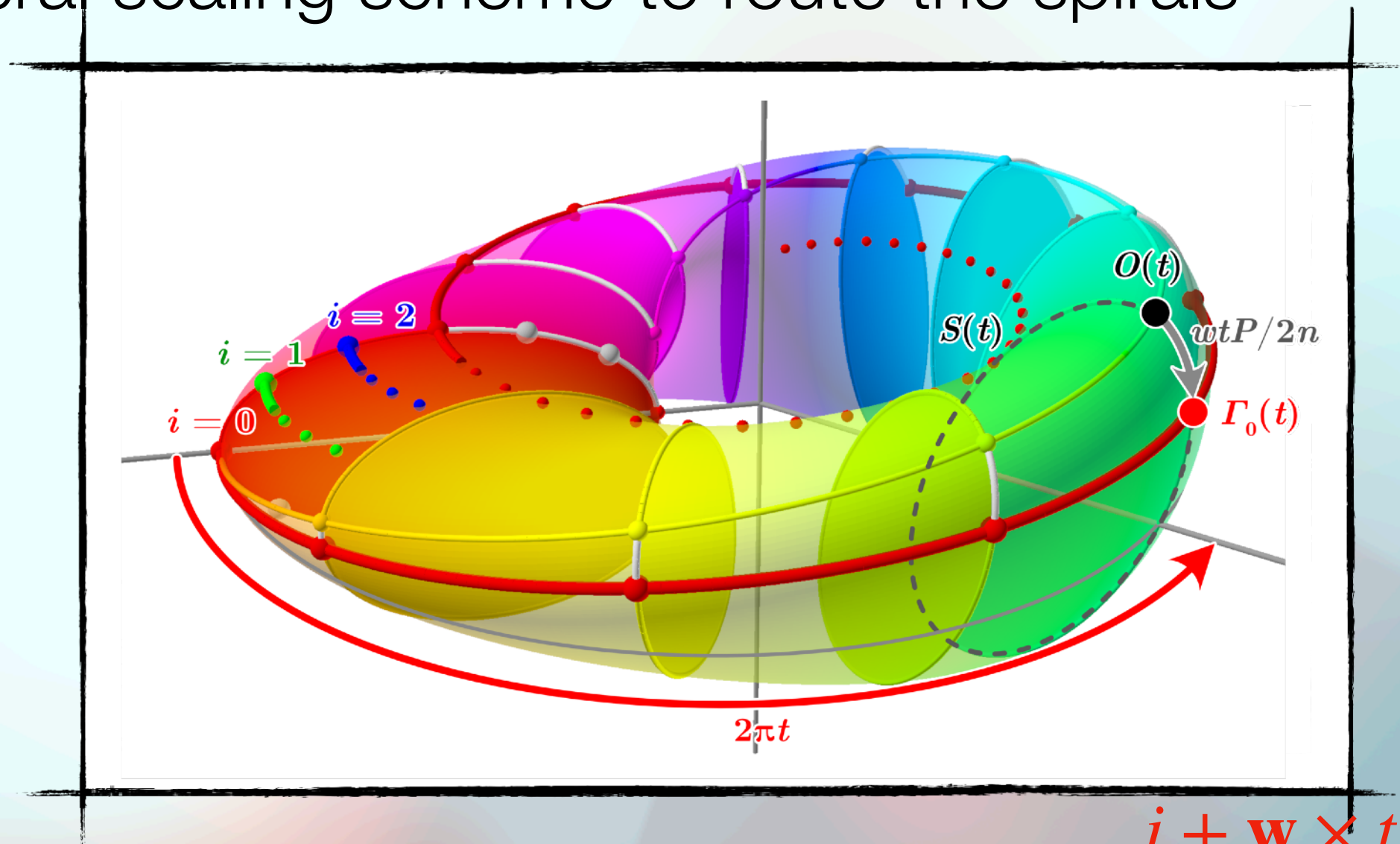
The case of tight curved shapes

A new spiral paradigm?



The case of tight curved shapes

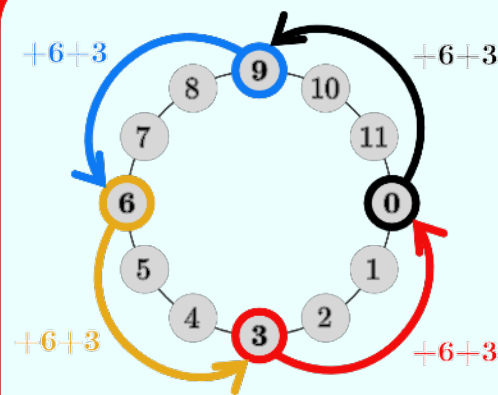
A general scaling scheme to route the spirals



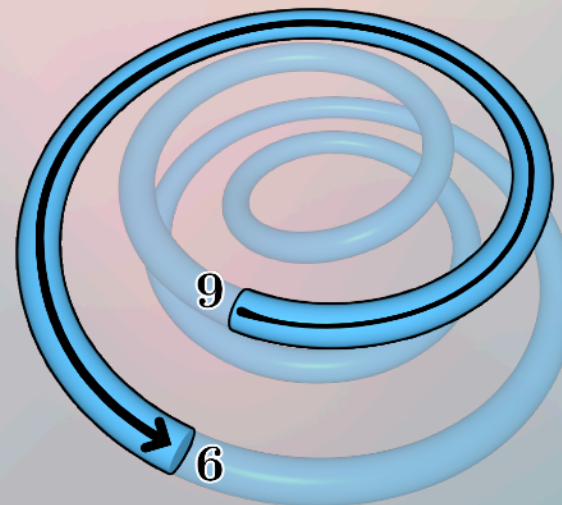
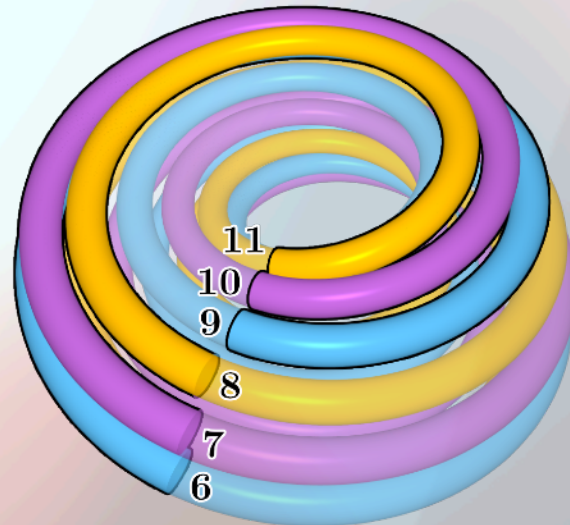
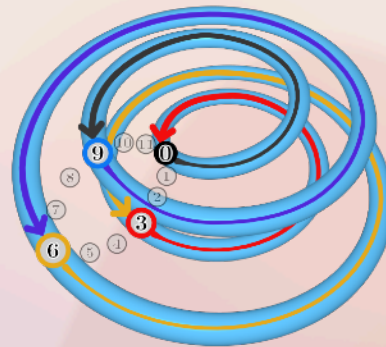
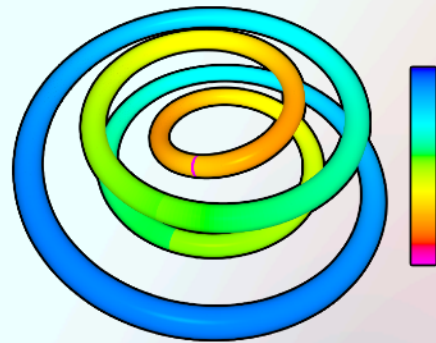
- Linear growth of the curvilinear abscissa: $s_i(t) = \frac{i + w \times t}{N} \times P$
where P is the section perimeter and N is the number of helices
- $w \in \mathbb{Z}$ is the winding parameter which determines the helices curvature

The case of tight curved shapes

Choose the winding parameter w



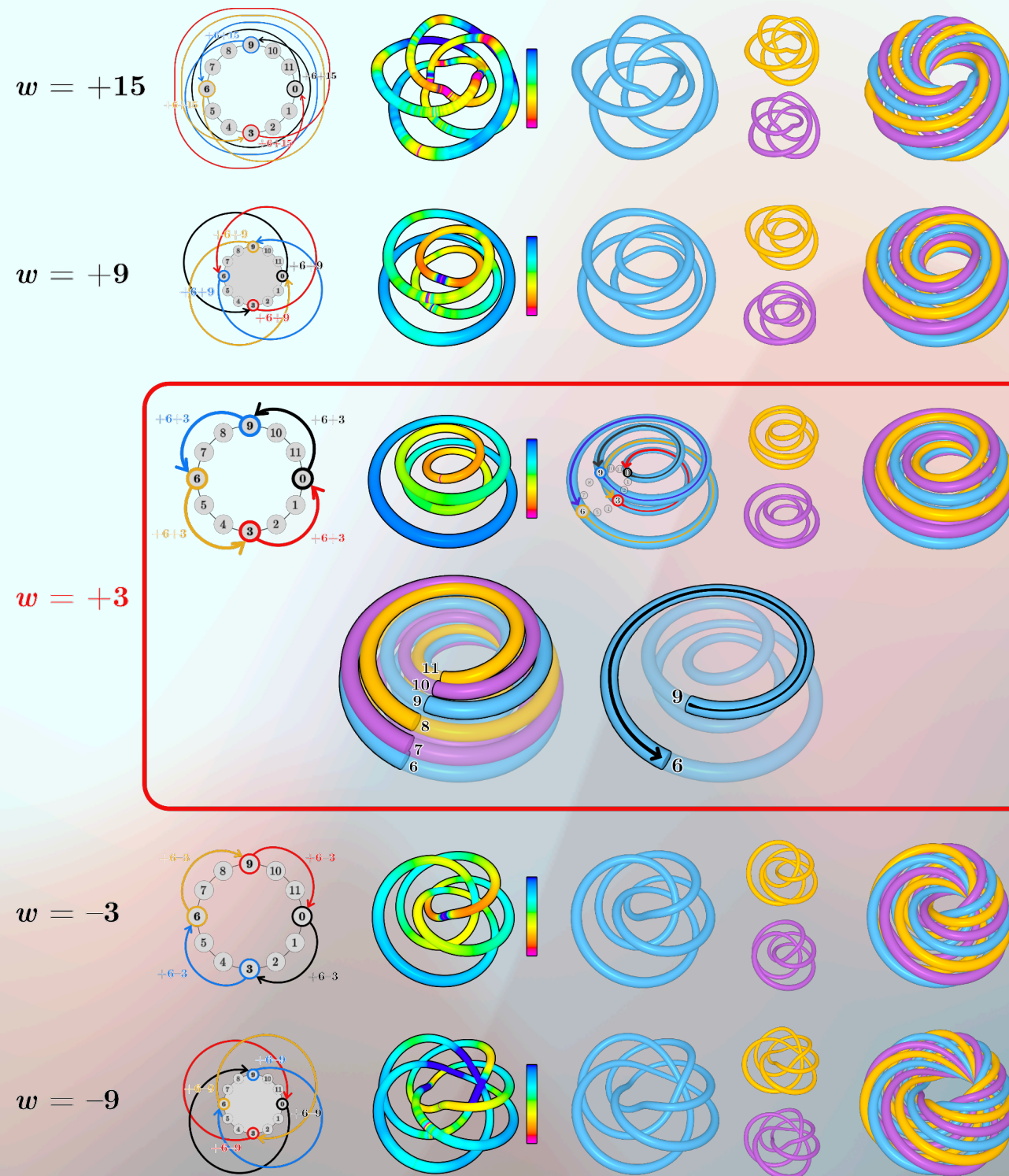
$$w = +3$$



$N = 12$ helices

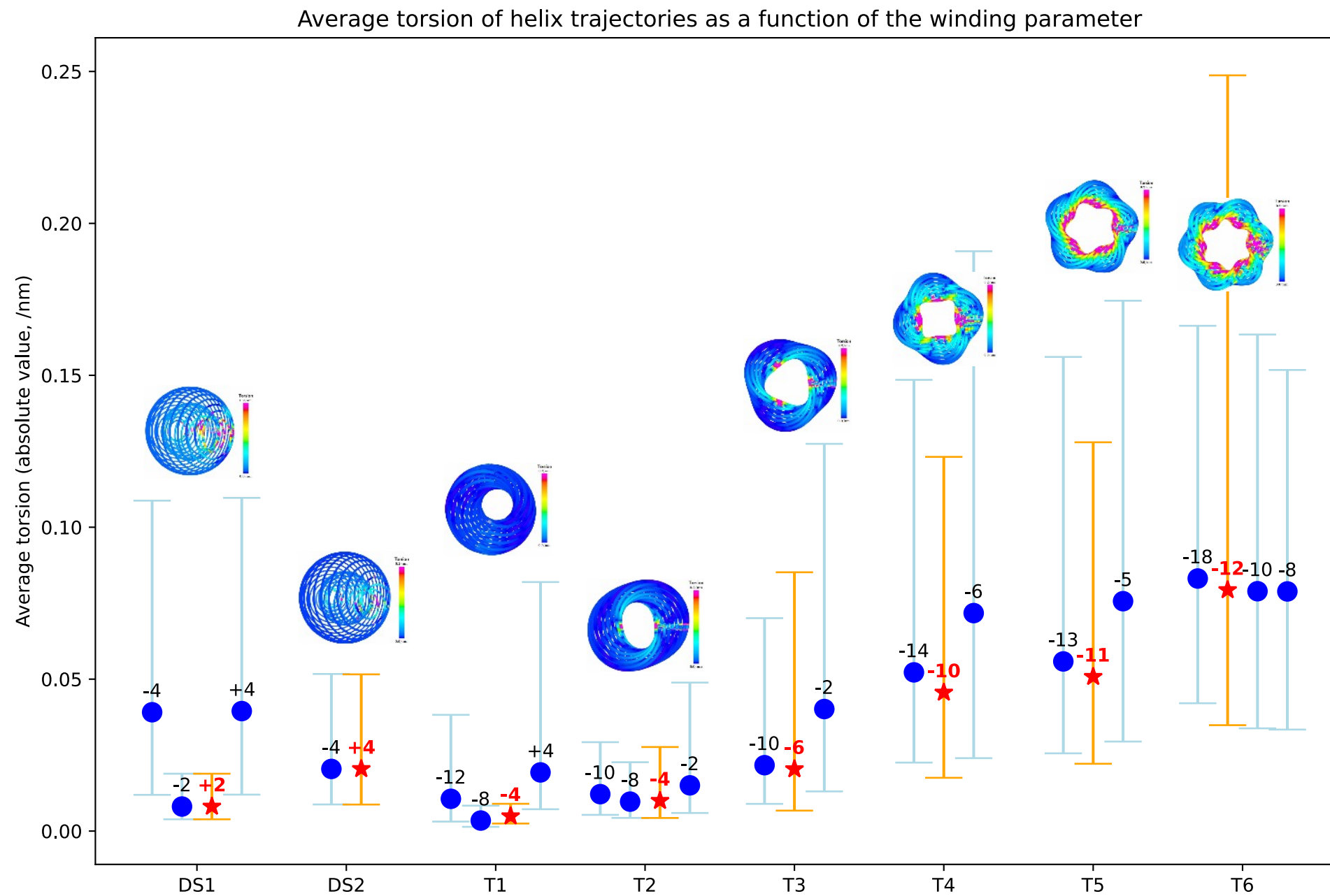
The case of tight curved shapes

Choose the winding parameter w



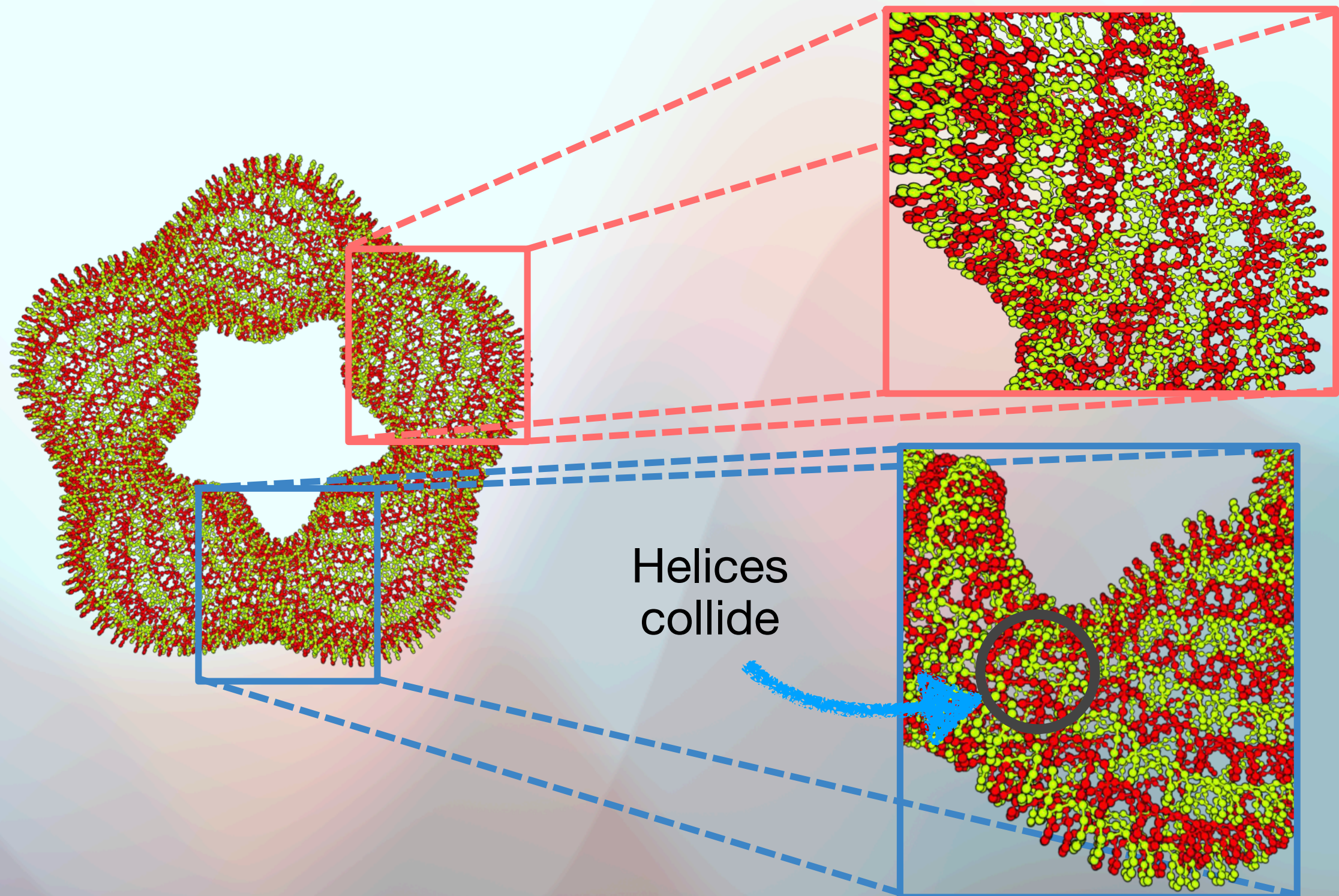
**Choosing
 $w = +3$
keeps the
helices
straight**

Winding parameter w controls helices torsion



The case of tight curved shapes

Balancing the inter helix distances

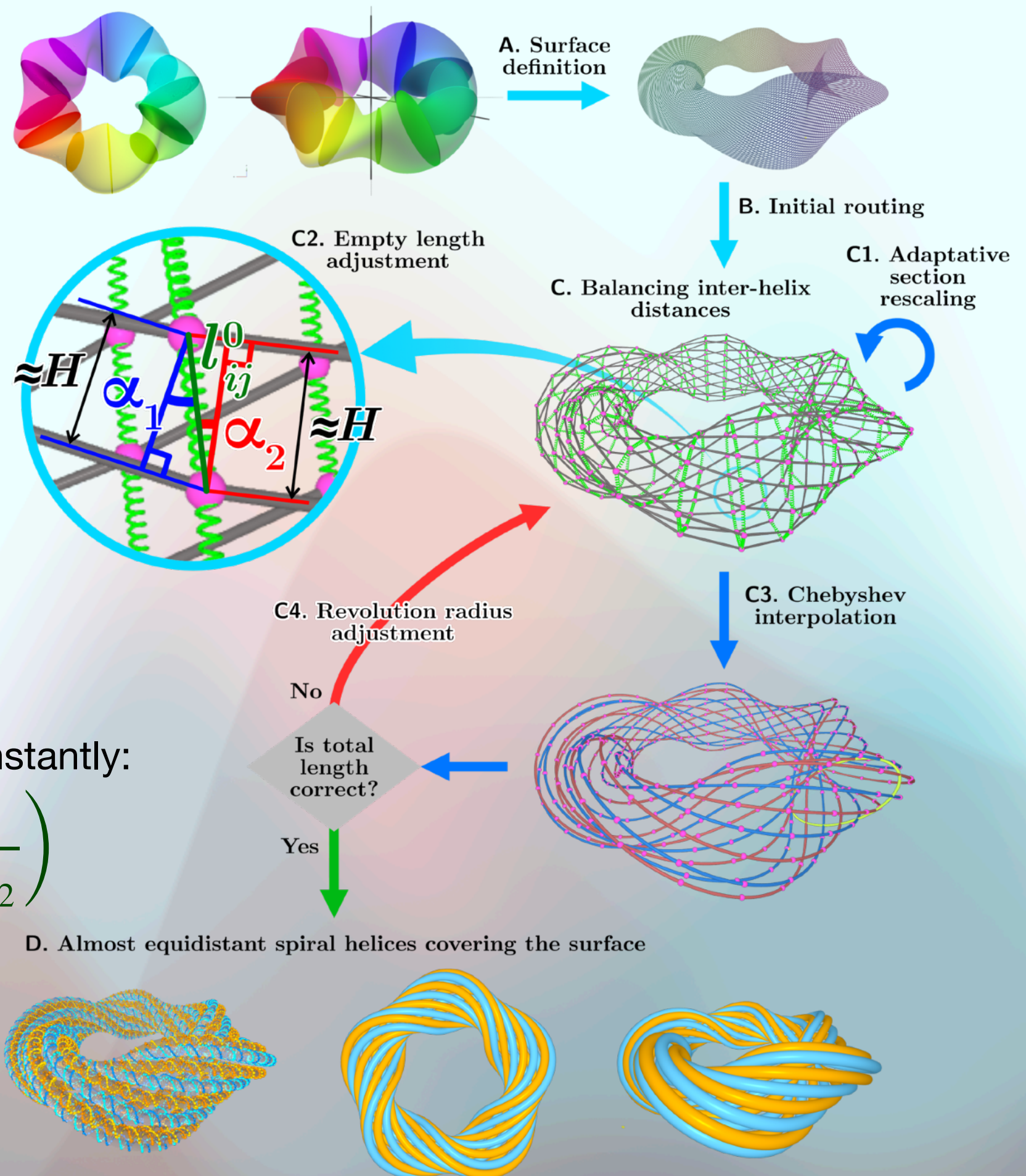


The case of tight curved shapes

Balancing the inter helix distances

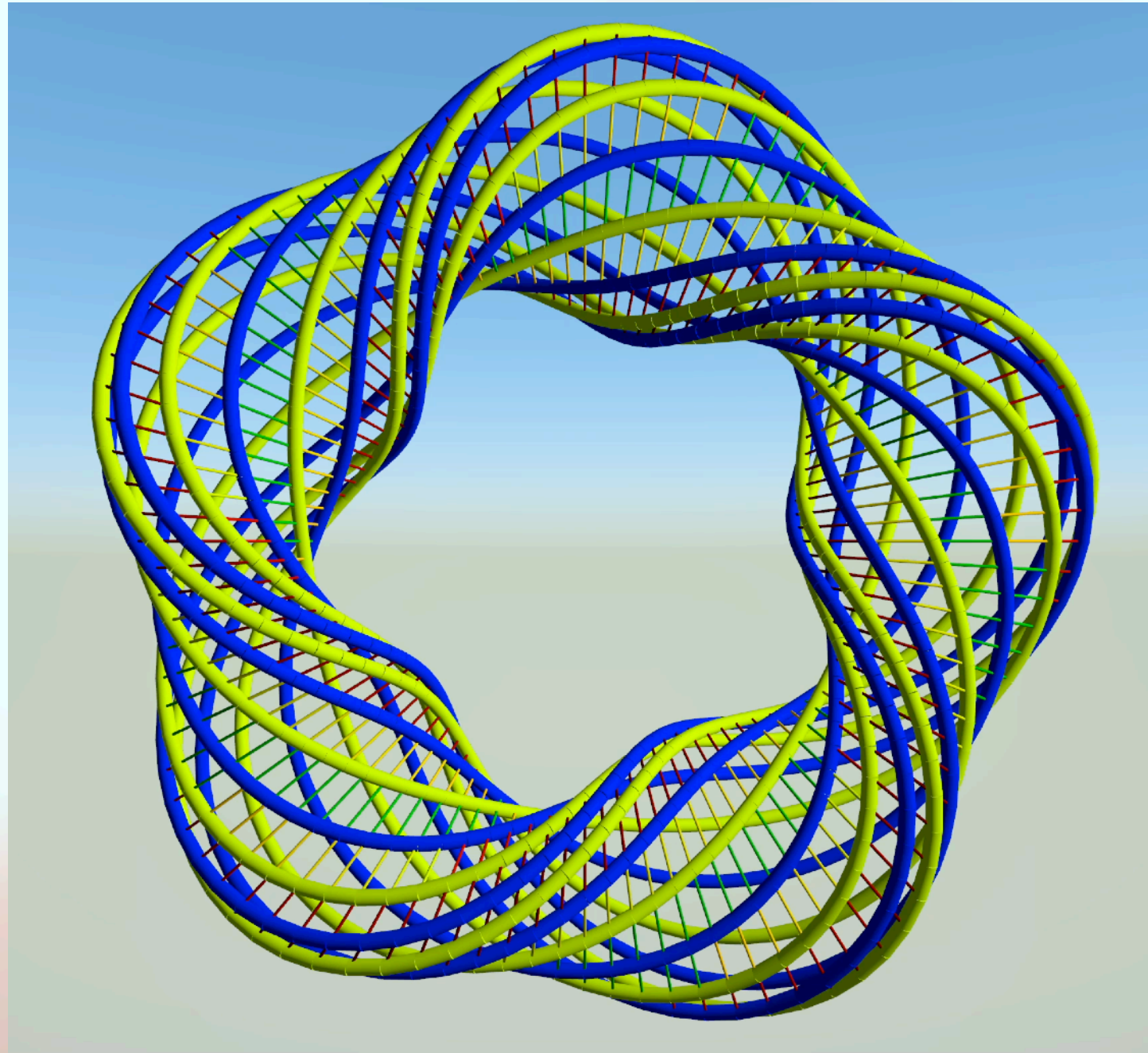
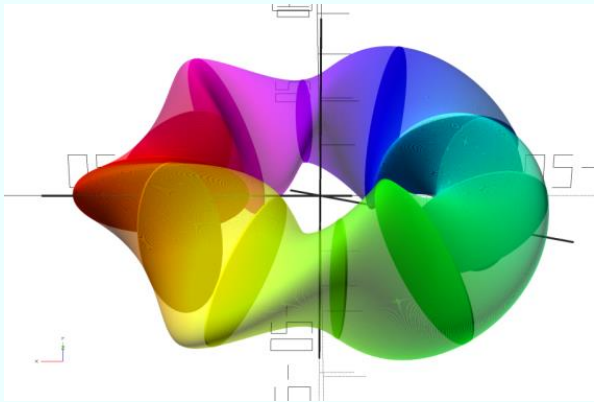
Update the empty lengths constantly:

$$l_{ij}^0 = \frac{1}{2} \left(\frac{H}{\cos \alpha_1} + \frac{H}{\cos \alpha_2} \right)$$



The case of tight curved shapes

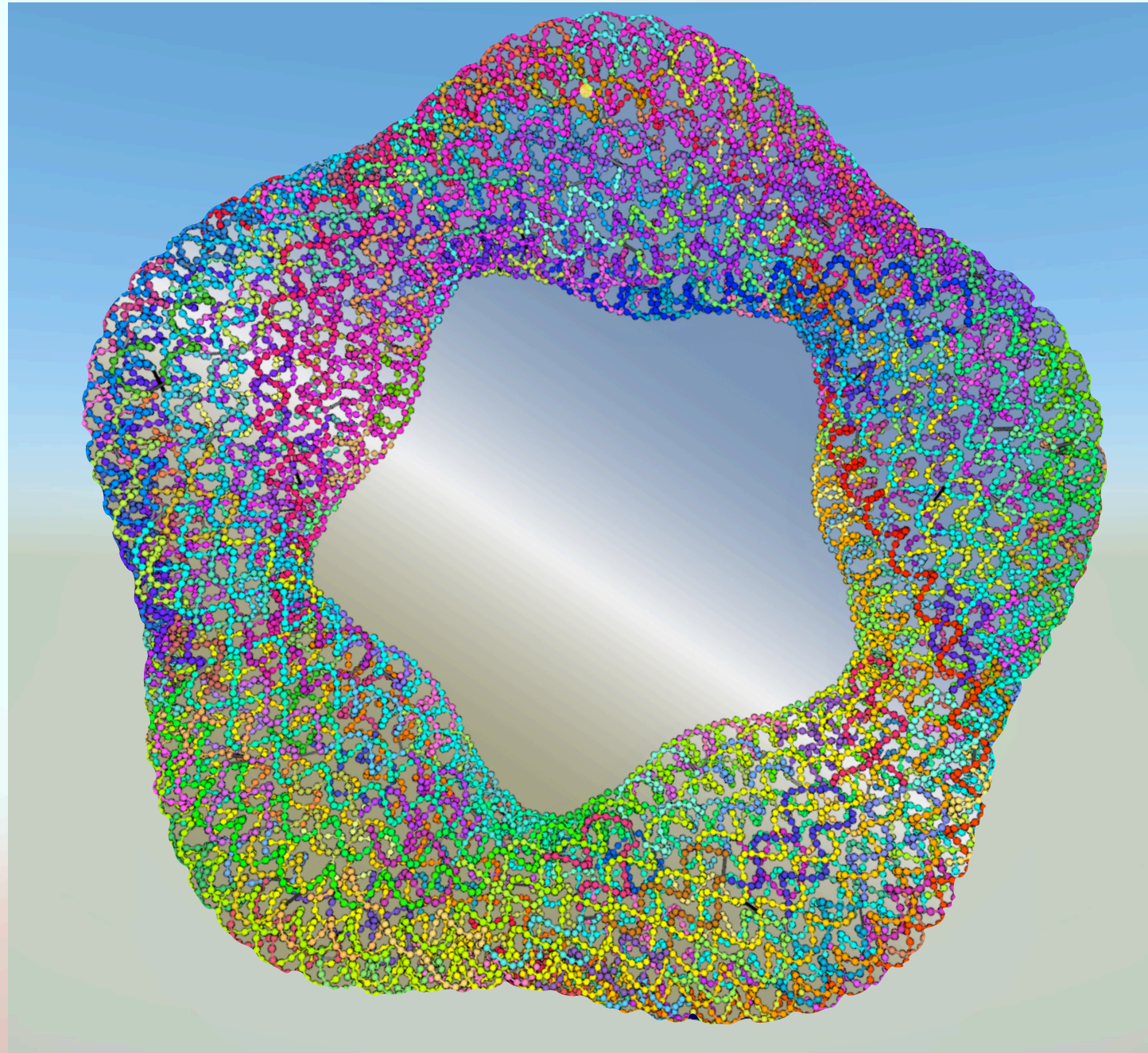
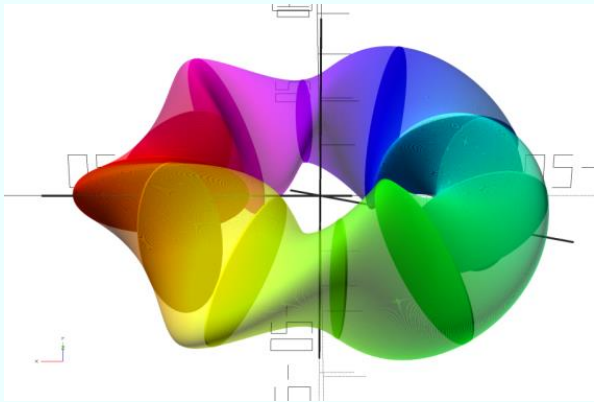
Balancing the inter helix distances



A relaxation process that outputs curves expressed as Чебышев polynomials whose length is exactly the desired scaffold length

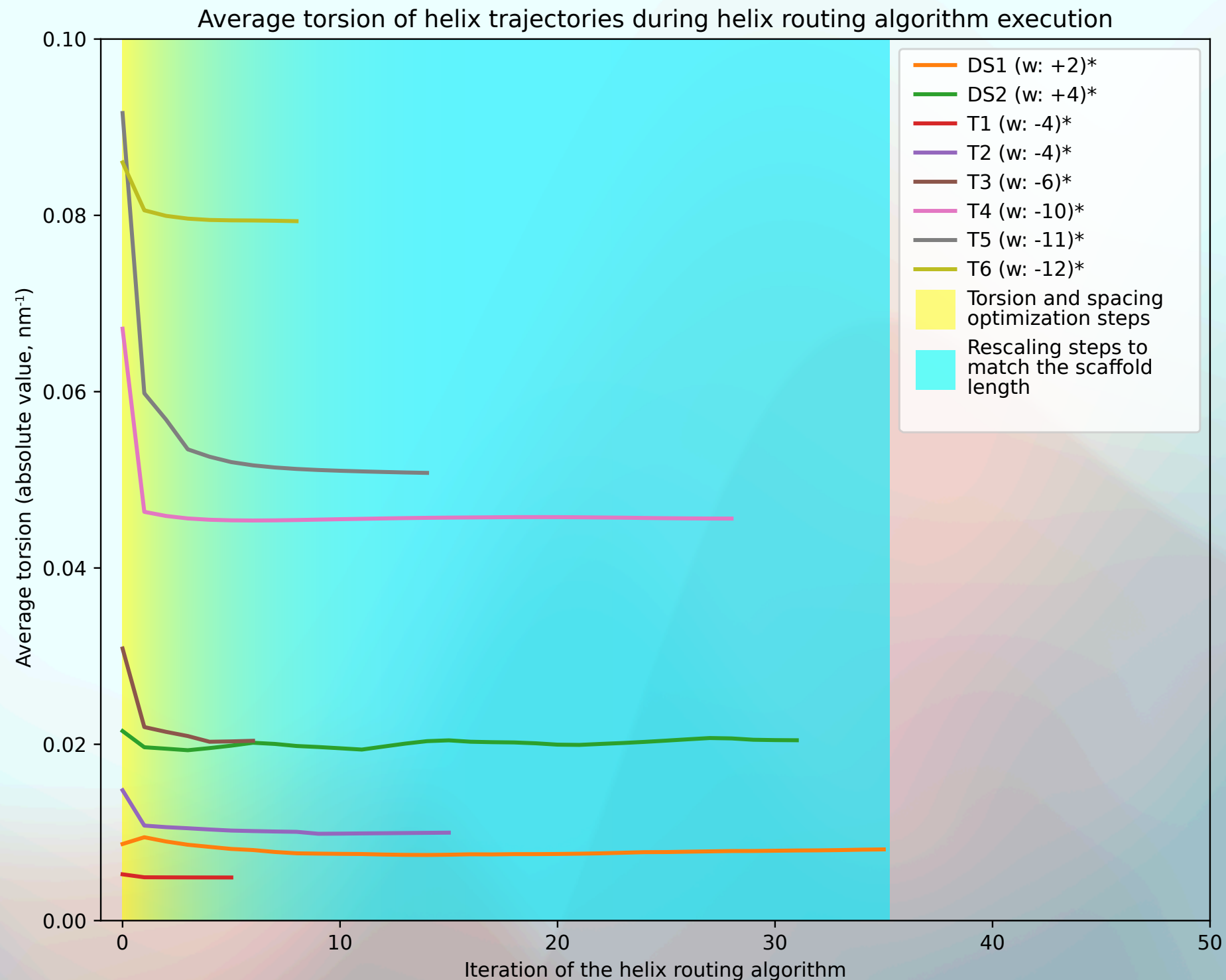
The case of tight curved shapes

Balancing the inter helix distances



A relaxation process that outputs curves expressed as Чебышев polynomials whose length is exactly the desired scaffold length

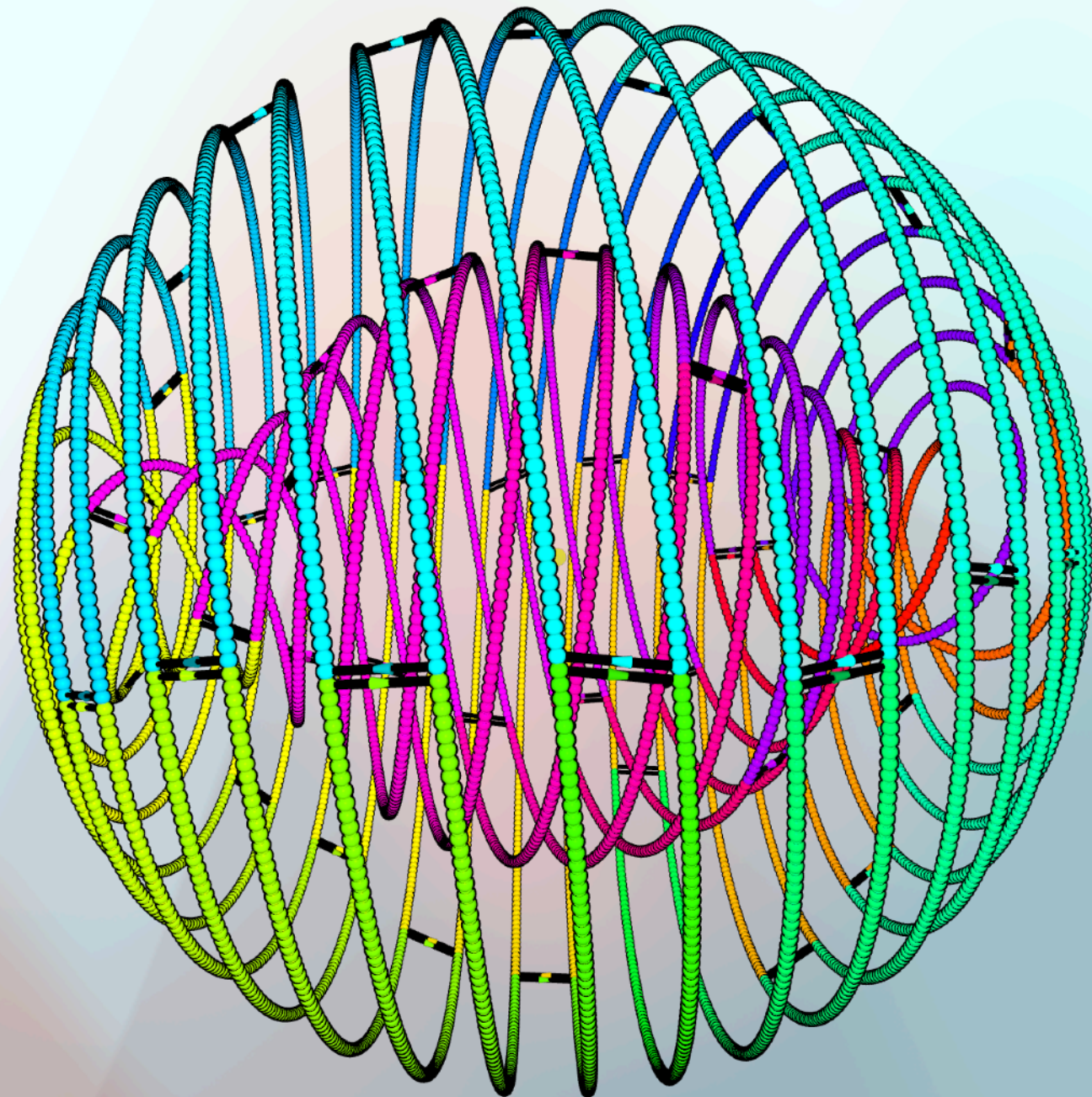
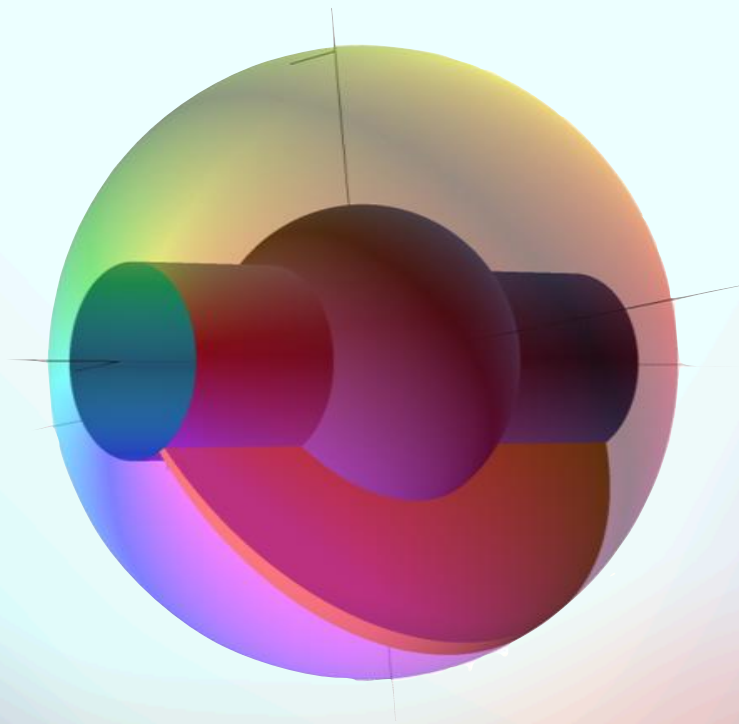
Routing algorithm deduces the torsion as well



The initial iteration reduces the torsion of the helices, the following ones mainly focus on scaling to match the scaffold length

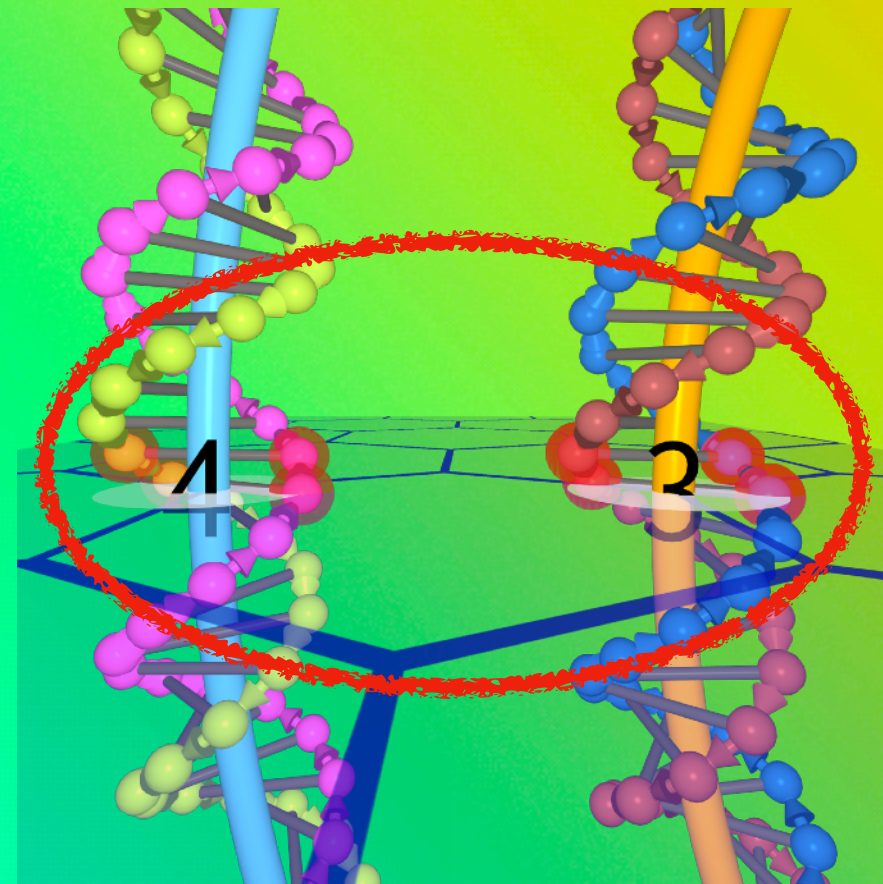
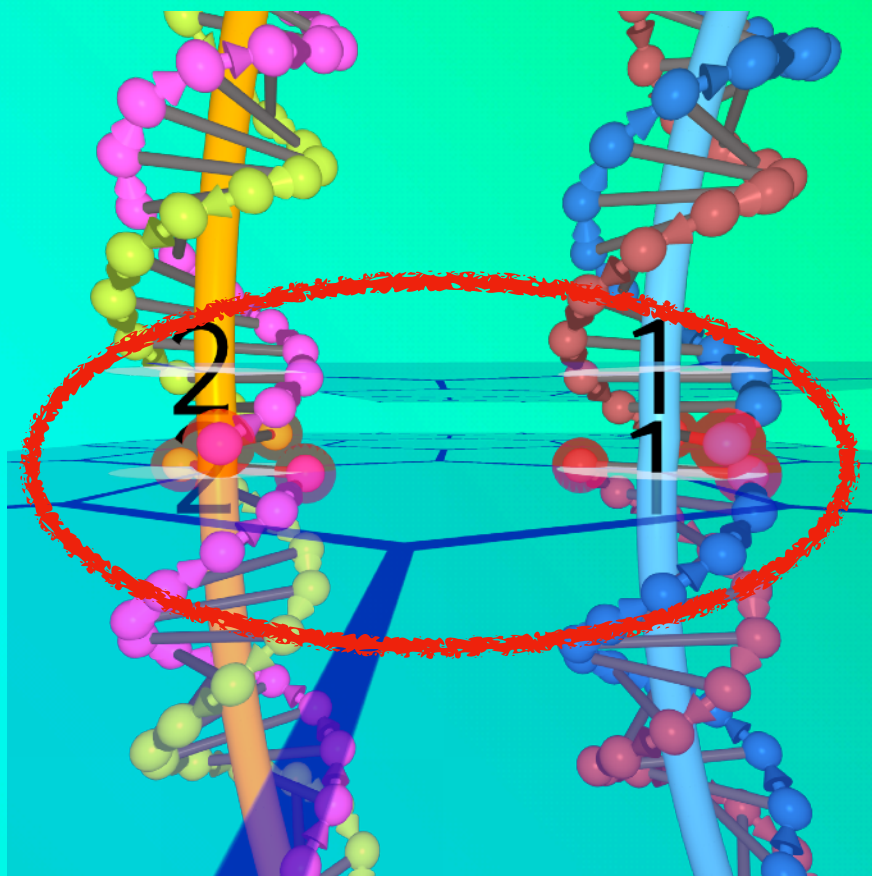
The case of tight curved shapes

Balancing the inter helix distances



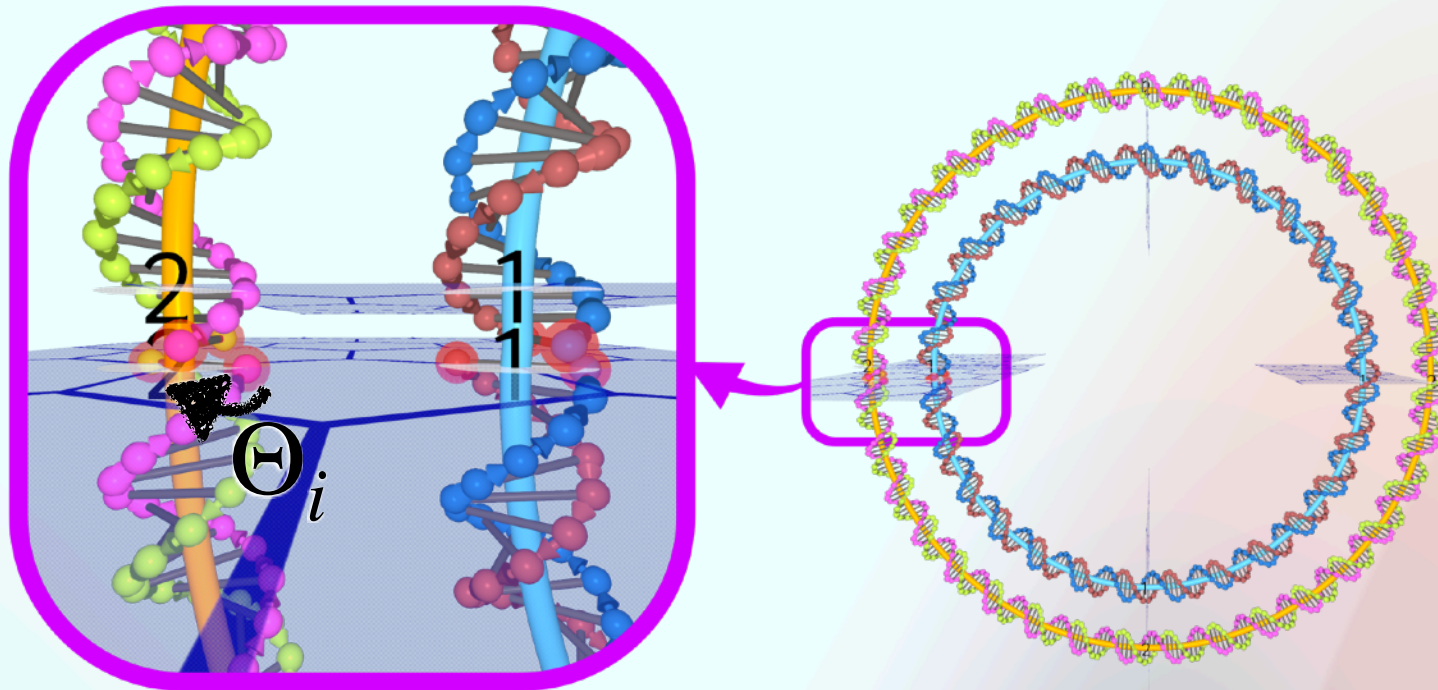
A relaxation process that outputs curves expressed as Чебышев polynomials whose length is exactly the desired scaffold length

2. Adjusting the DNA parameters

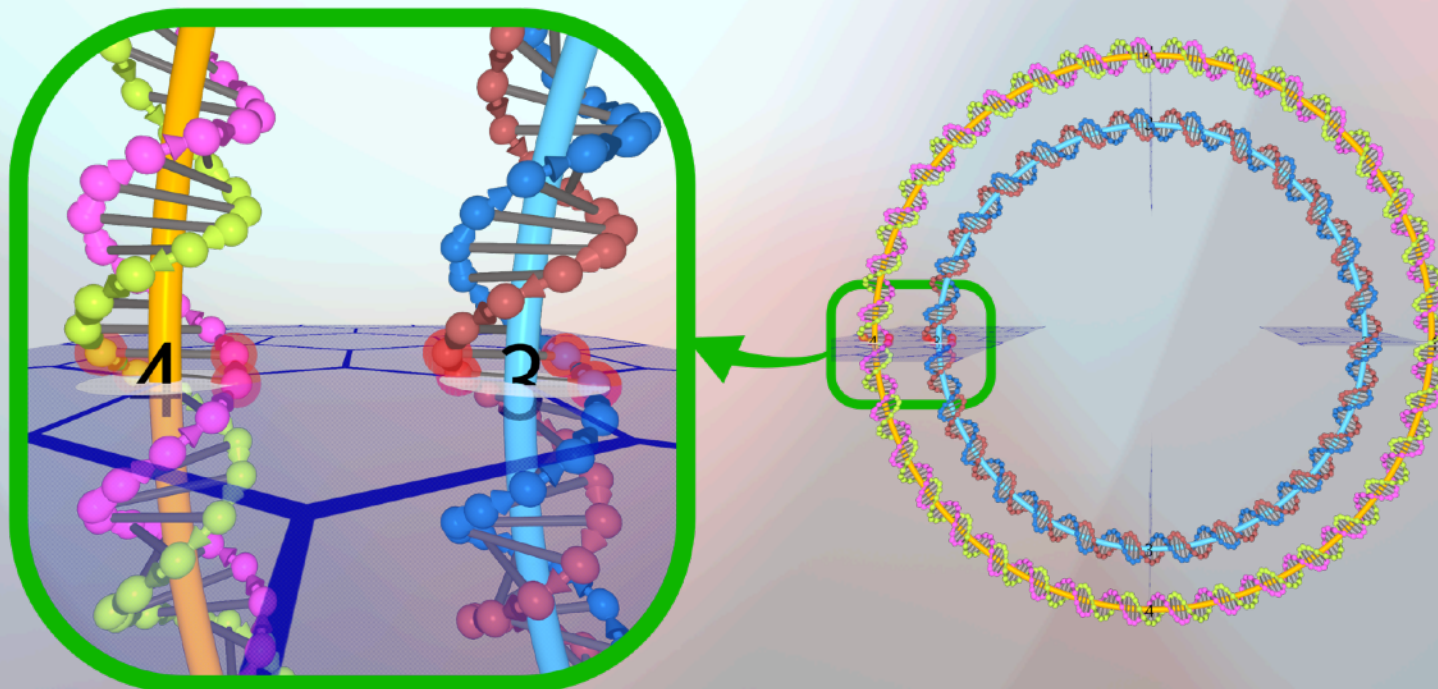


Adjusting DNA parameters

Adjusting DNA parameter to close planar curved helices



Missaligned



Aligned

Close the helices:

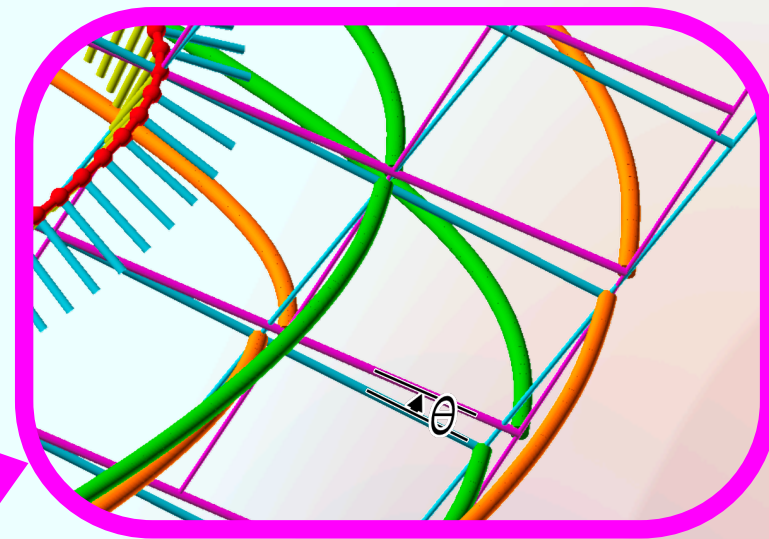
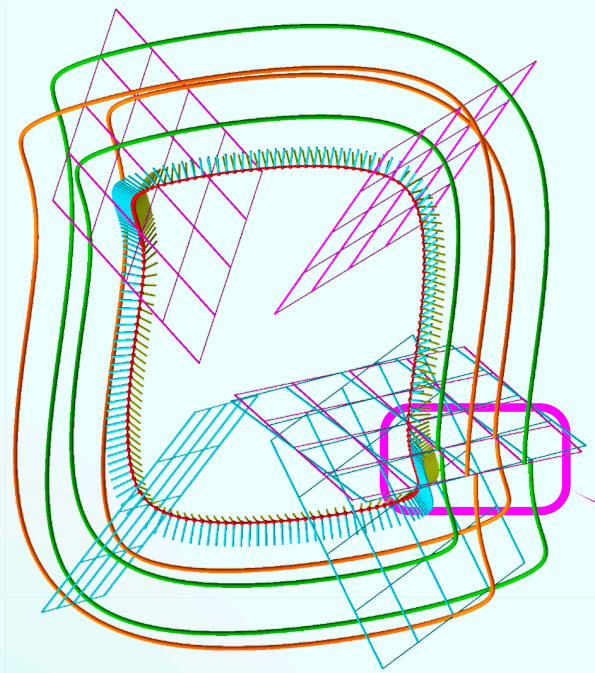
Compute iteratively the frames along each helix and **adjust minimally the twist** of each helix so that both ends of each helix coincide:

$$\beta_i := \frac{2\pi}{10.44} + \frac{\Theta_i}{N_i}$$

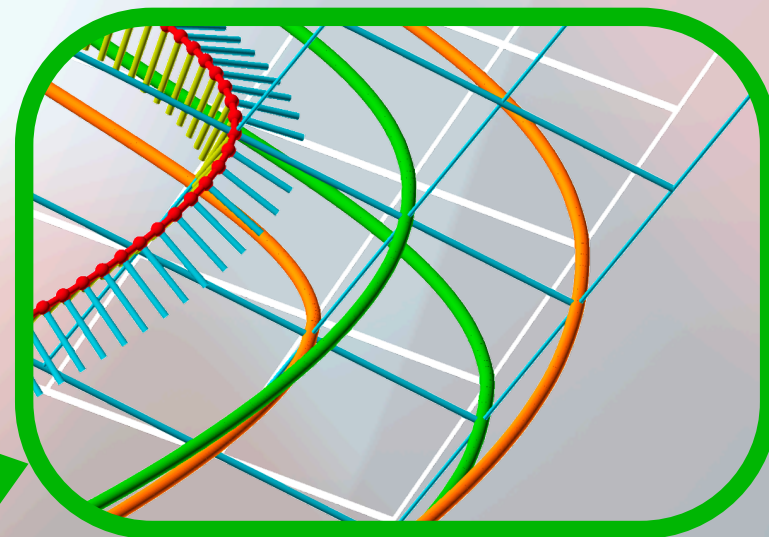
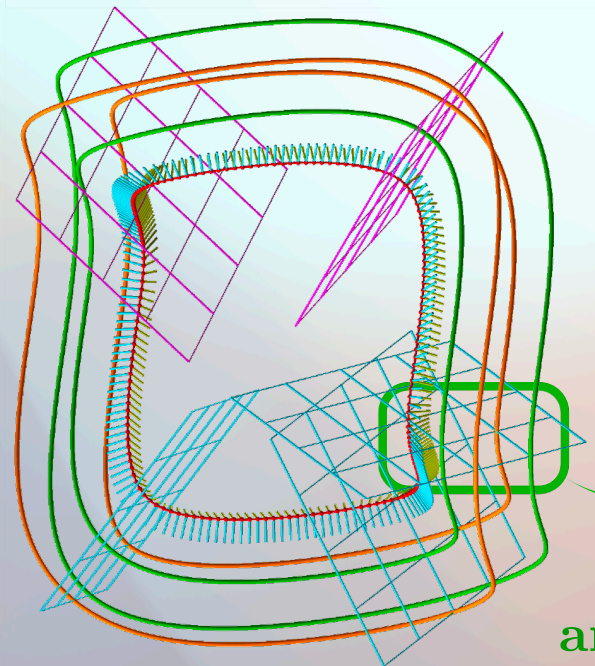
This spreads the strain

Adjusting DNA parameters

Adjusting DNA parameters for curved DNA bundle



The ends of the trajectories are misaligned



We align minimally the initial and final frames to align the trajectories

Closing the helices:

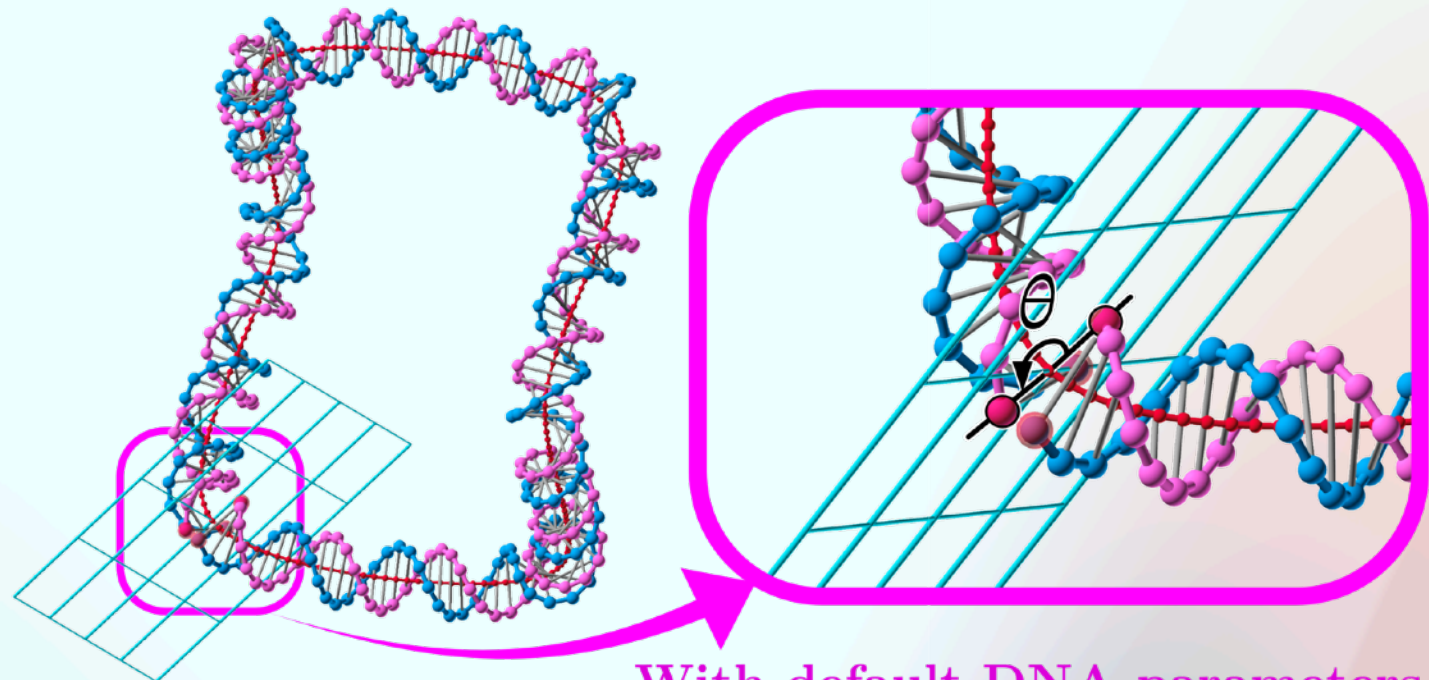
1) Compute iteratively the frames along the curve and **adjust minimally the twist of the grid** and then

2) Adjust minimally the **twist** of each helix so that both ends of each helix coincide:

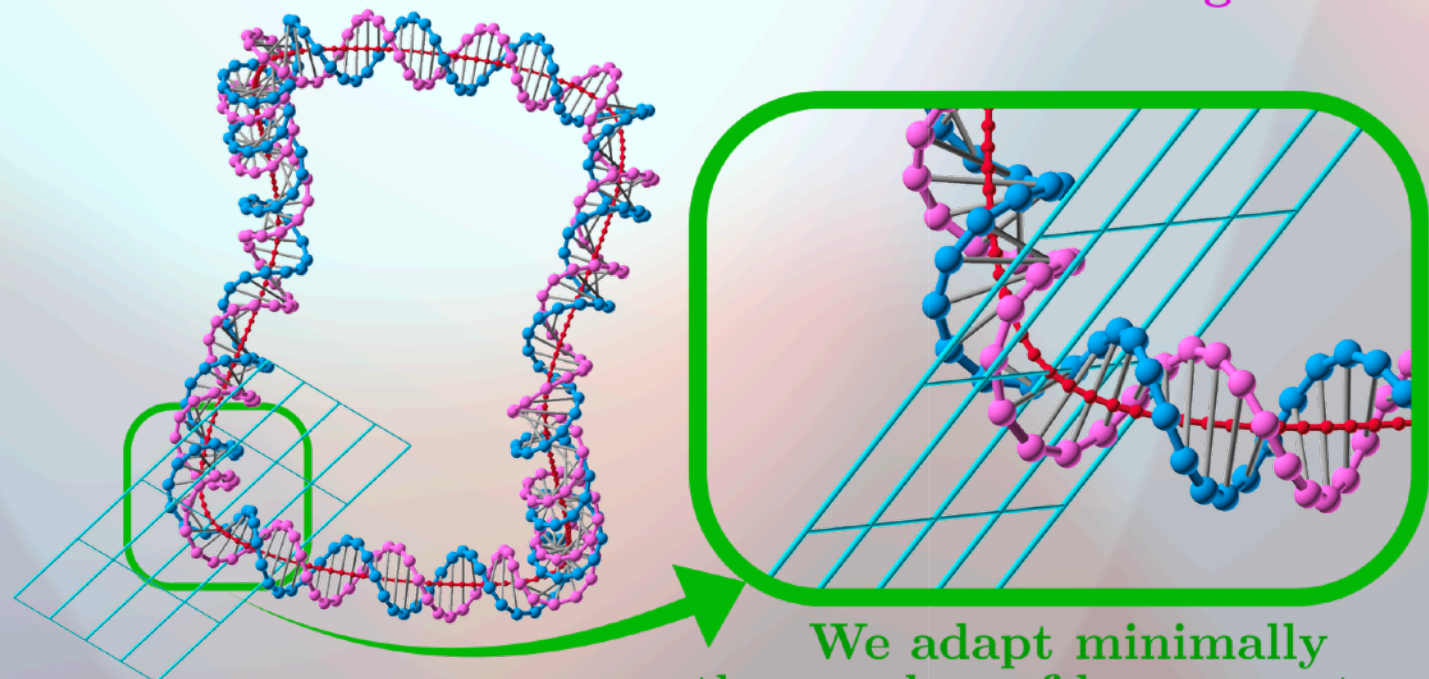
$$\beta_i := \frac{2\pi}{10.44} + \frac{\Theta_i}{N_i}$$

Adjusting DNA parameters

Adjusting DNA parameters for curved DNA bundle



With default DNA parameters,
strands are misaligned



We adapt minimally
the number of bases per turn
to align the strands

Closing the helices:

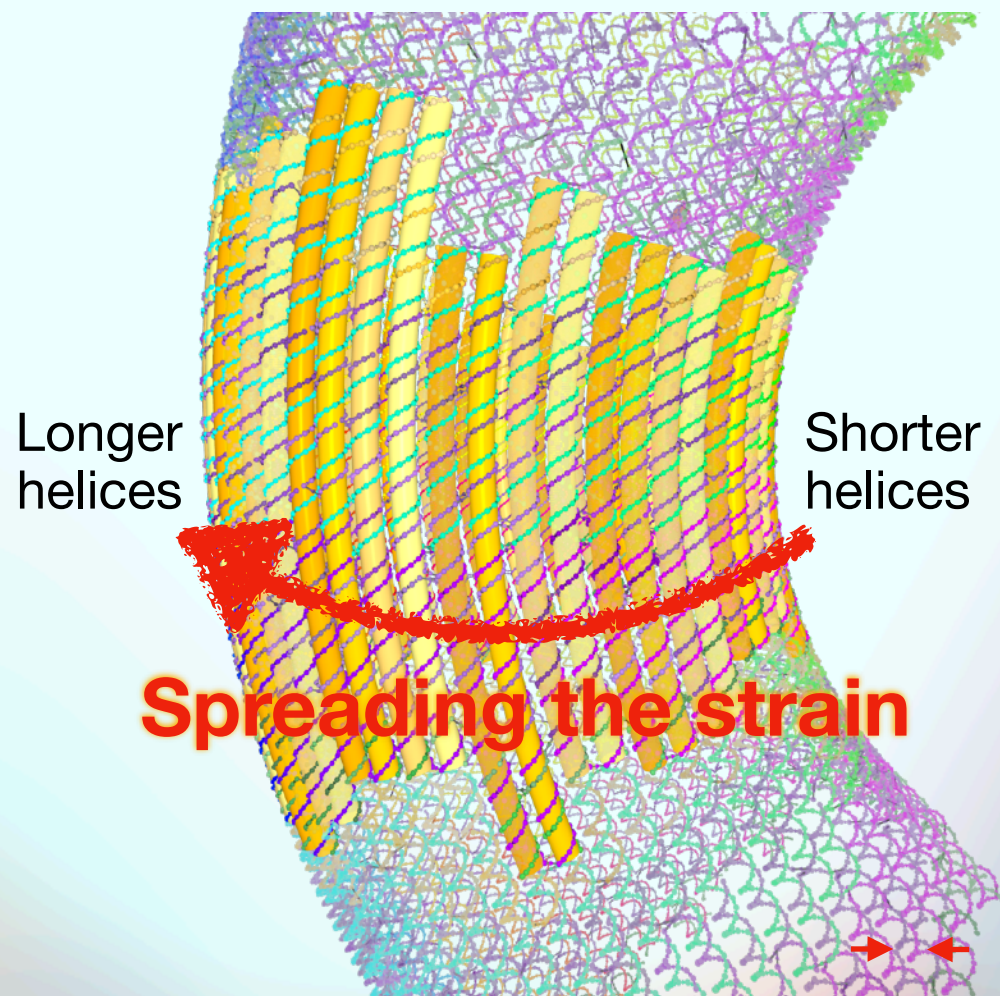
- 1) Compute iteratively the frames along the curve and **adjust minimally the twist of the grid** and then
- 2) **Adjust minimally the twist** of each helix so that both ends of each helix coincide:

$$\beta_i := \frac{2\pi}{10.44} + \frac{\Theta_i}{N_i}$$

**The correction is $O(1/N)$
few % for $\geq 100\text{bp}$ -long helices**

Adjusting DNA parameters

Spreading the constraints on short helices



Fix bounds on the rise and twist, e.g.

rise $\in [0.32, 0.35]$ nm / base pair

twist $\in [10.2, 10.8]$ base pairs / turn

For all helices that do not meet the bounds:

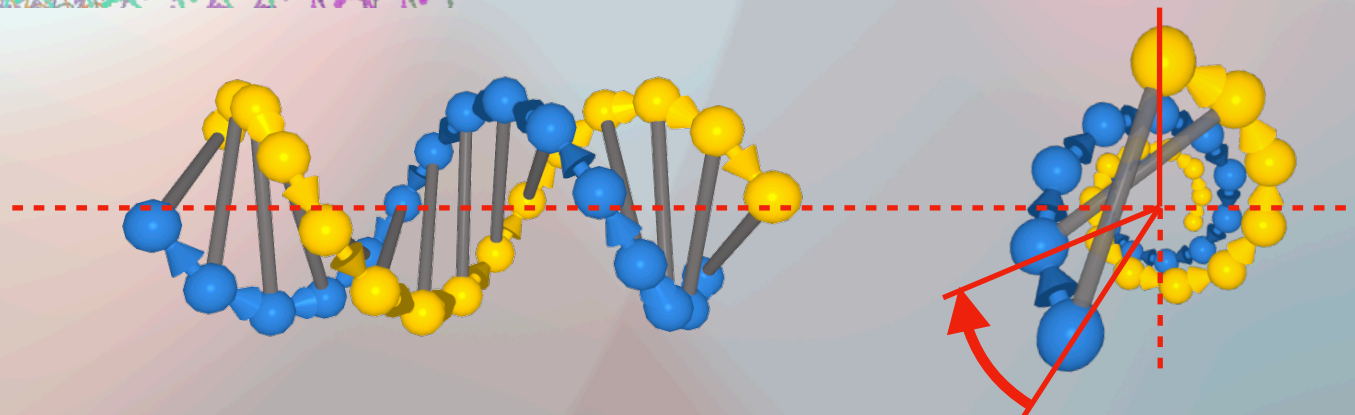
Look for the best variation ΔN so that

$$\widetilde{\text{rise}} = \frac{\text{Length in nm}}{N + \Delta N}, \quad \widetilde{\beta} = \frac{2\pi}{10.44} + \frac{\Theta_{\Delta N}}{N + \Delta N} \text{ and}$$

$\widetilde{\text{twist}} = 2\pi / \widetilde{\beta}$ are within the bounds

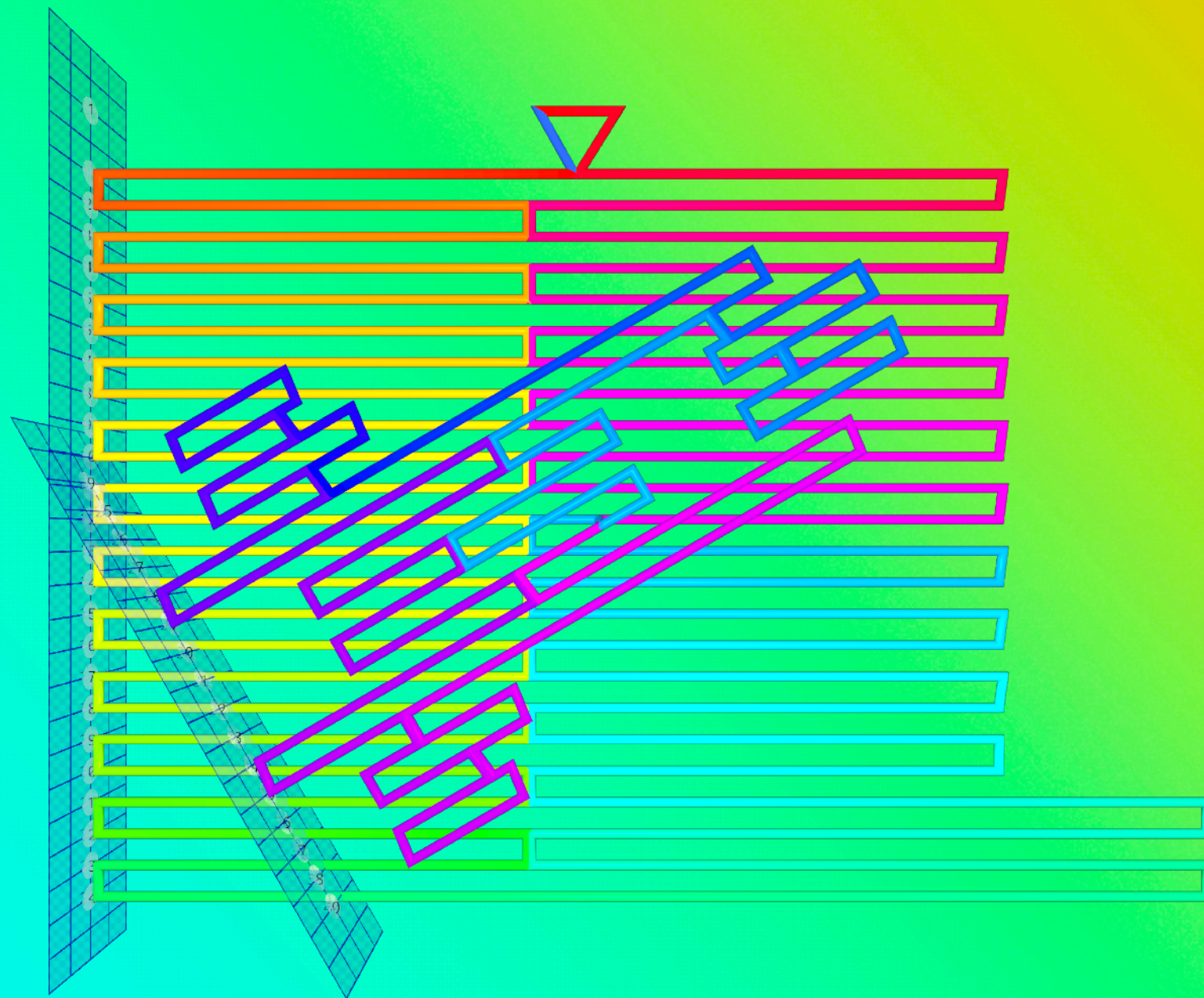
and spread the ΔN over the other helices

until all helices meet the bounds

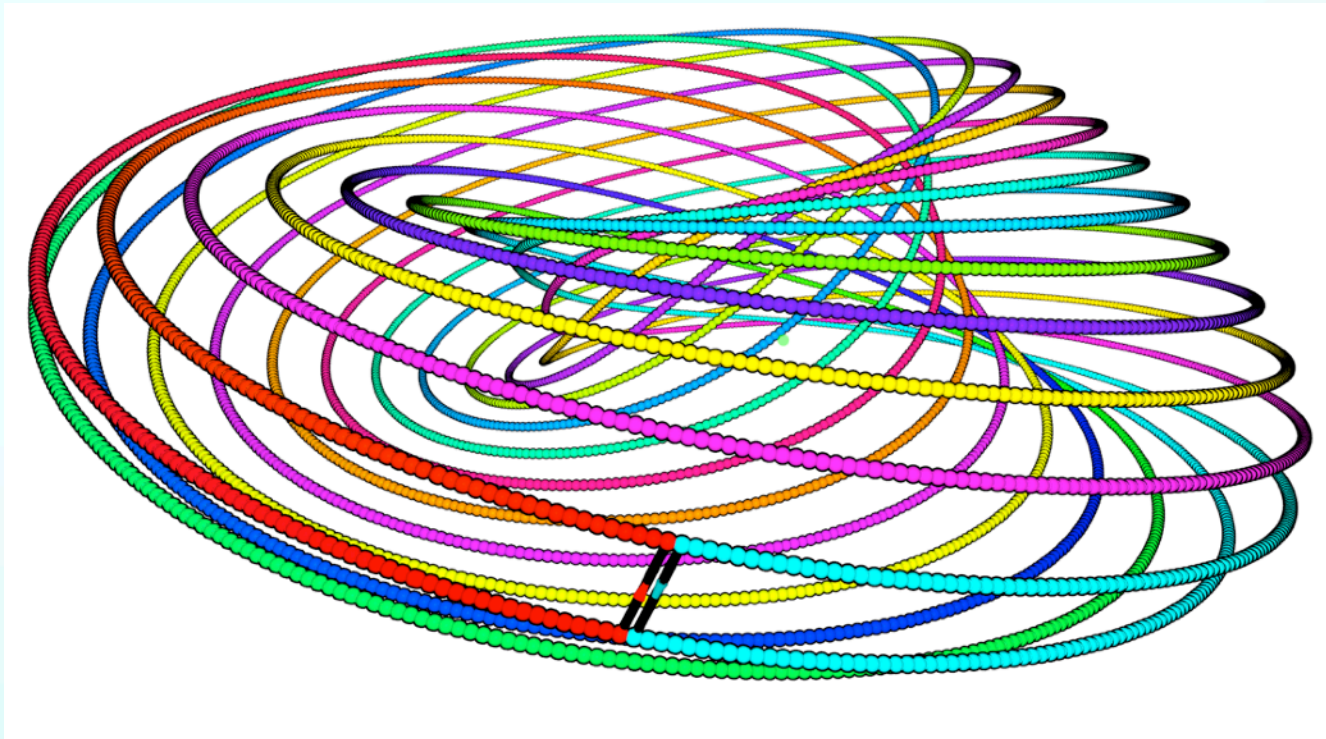


Changing the number of nucleotides N can help align the ends of the helix

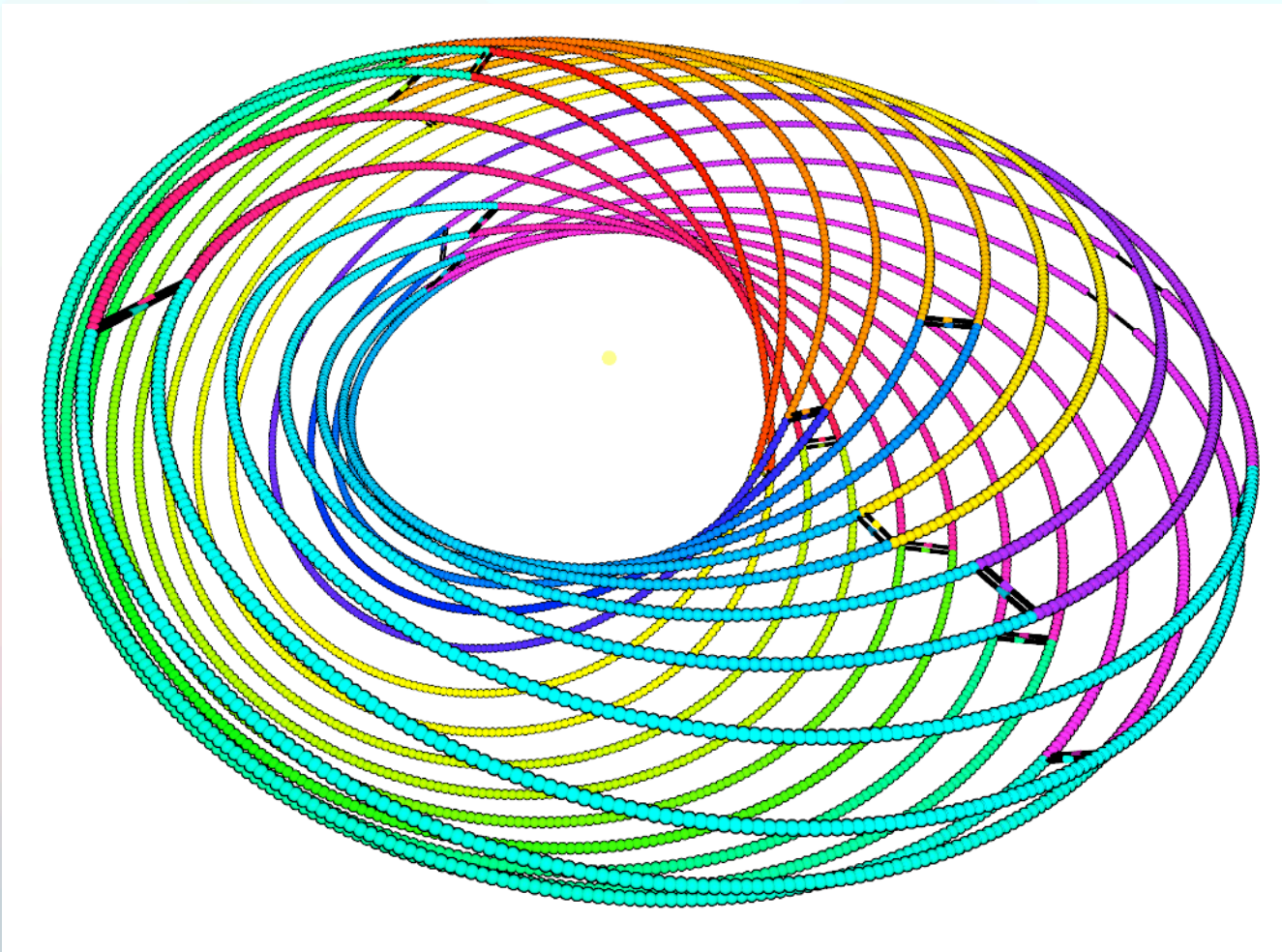
3. Routing the scaffold



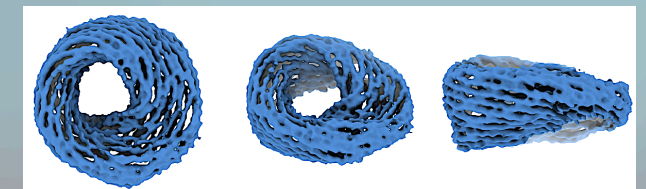
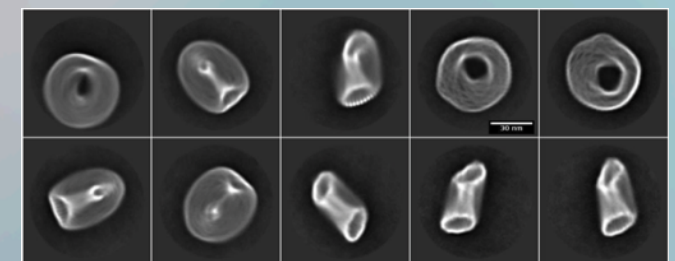
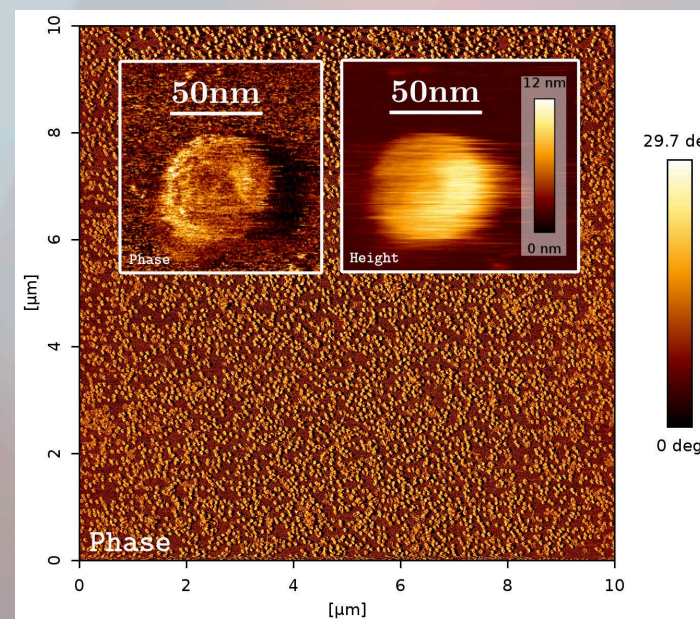
The scaffold route matters



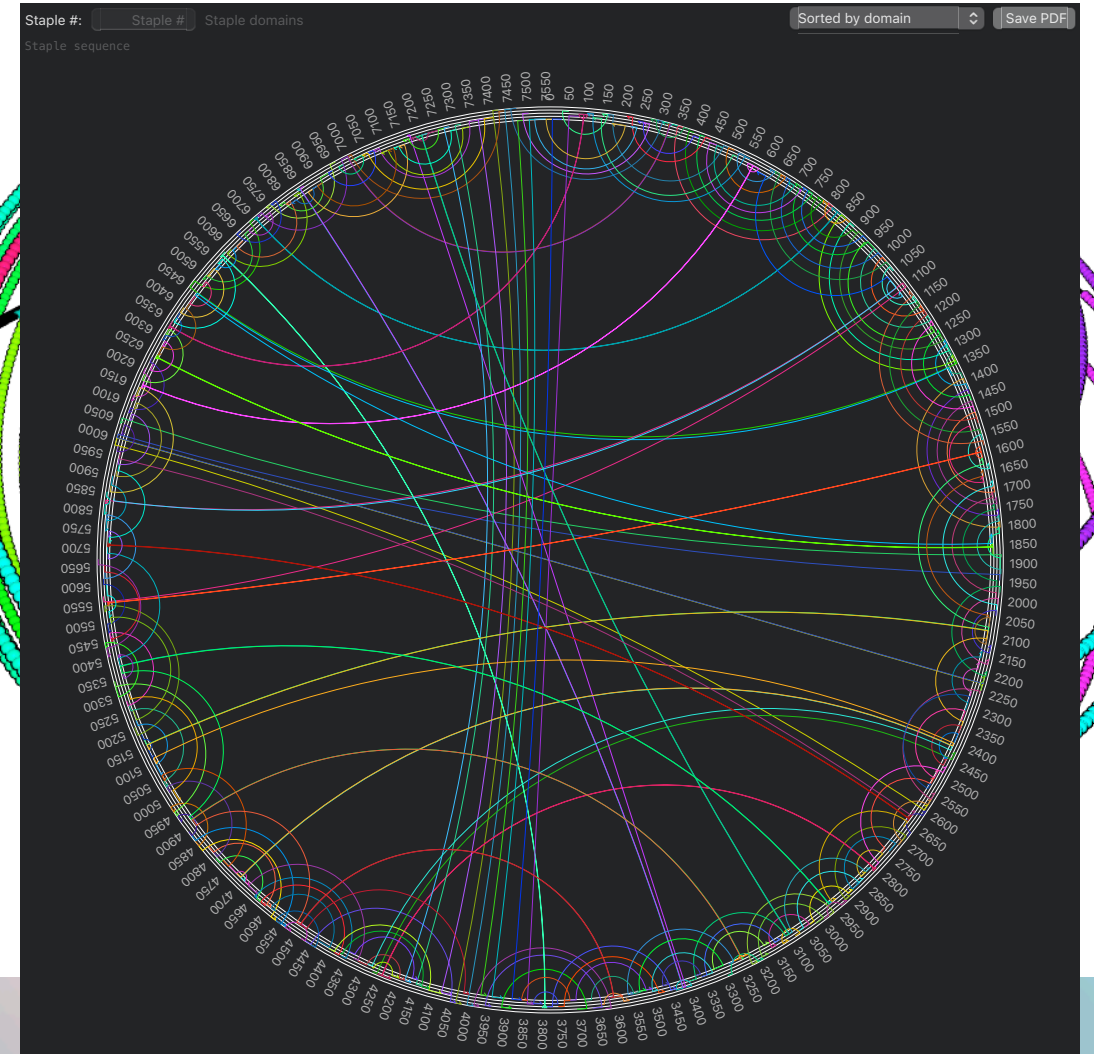
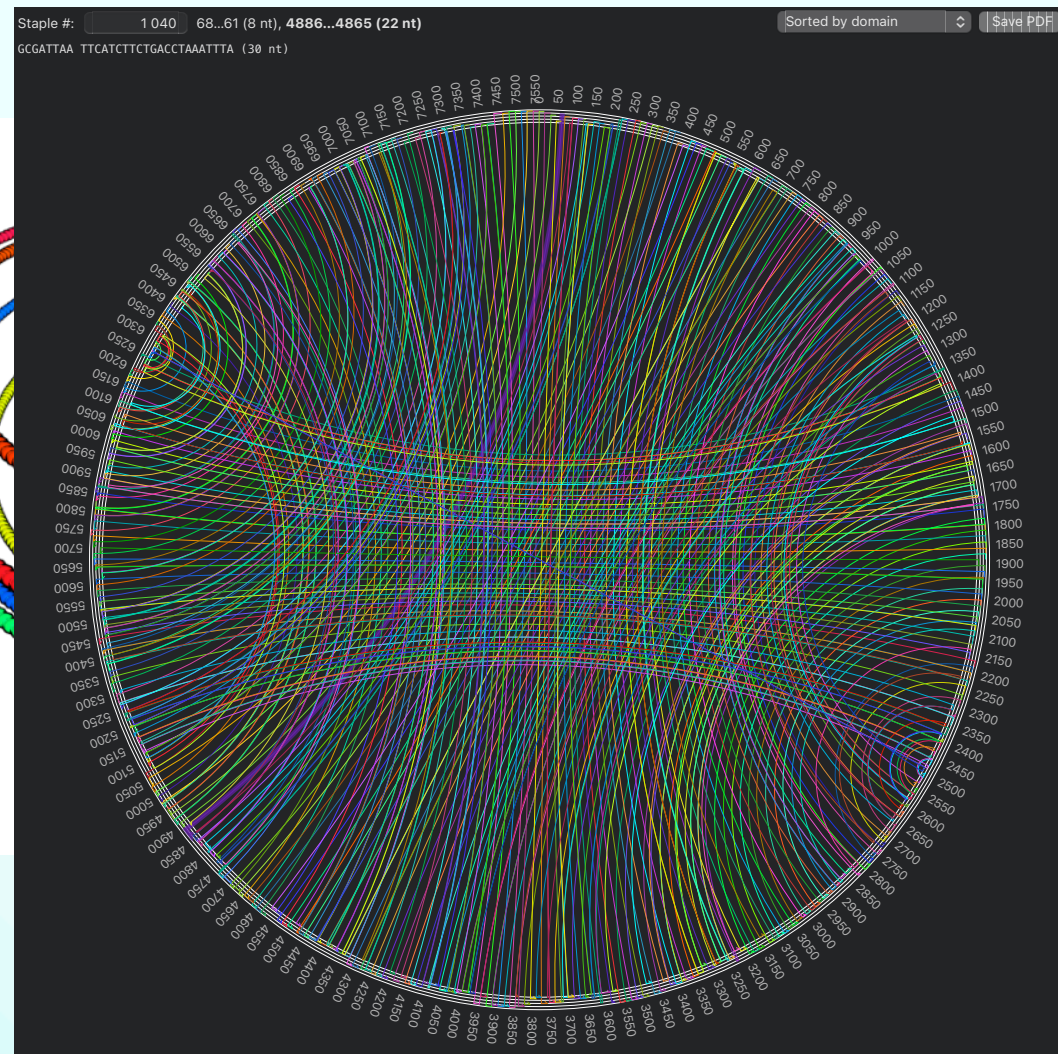
Fails to fold



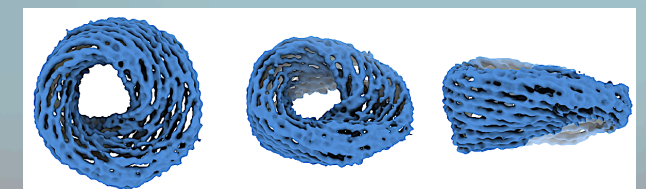
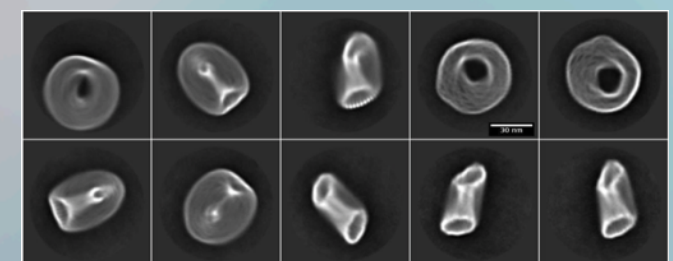
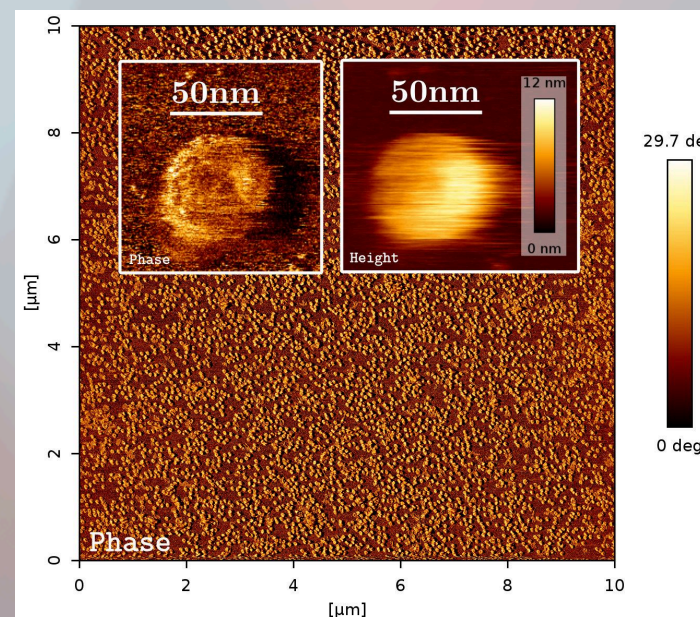
Folds!



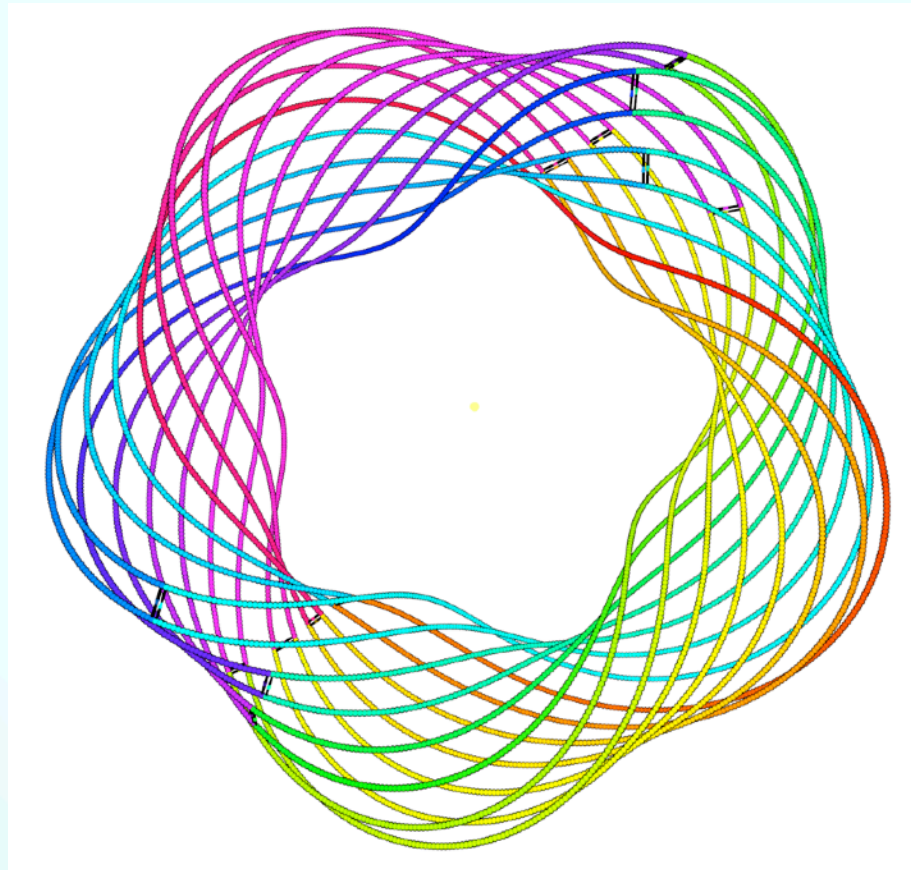
The scaffold route matters



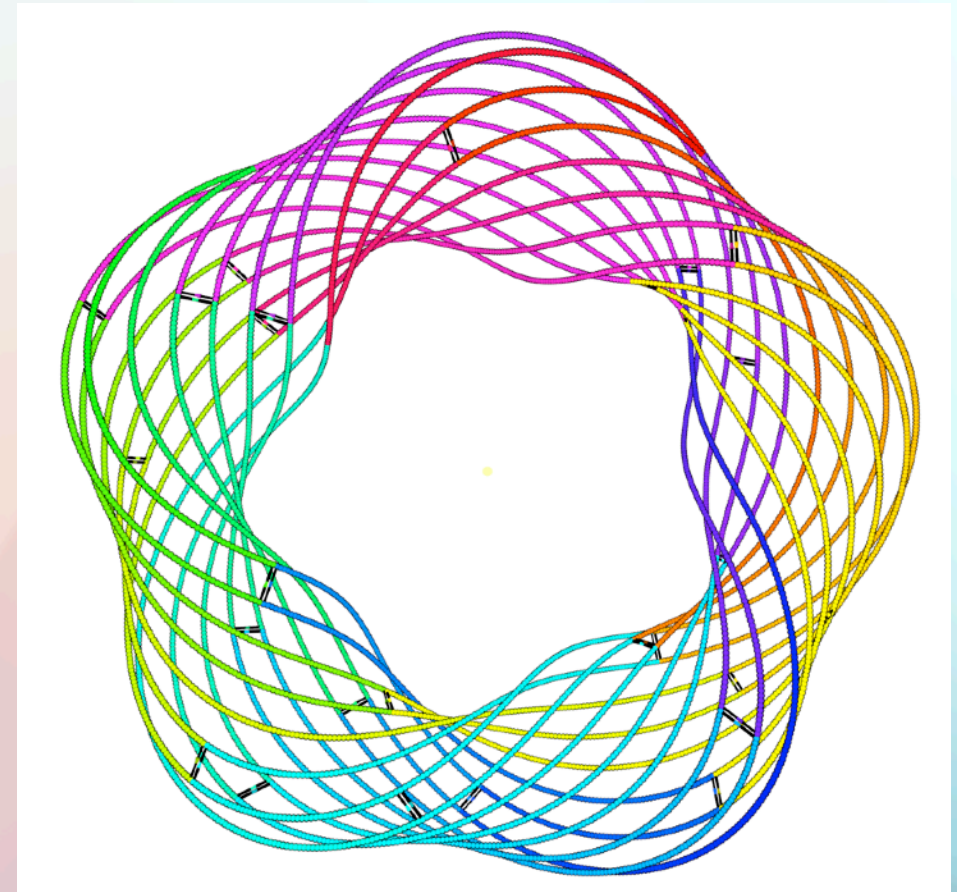
must cut to avoid
too long domains in the scaffold



The scaffold route matters



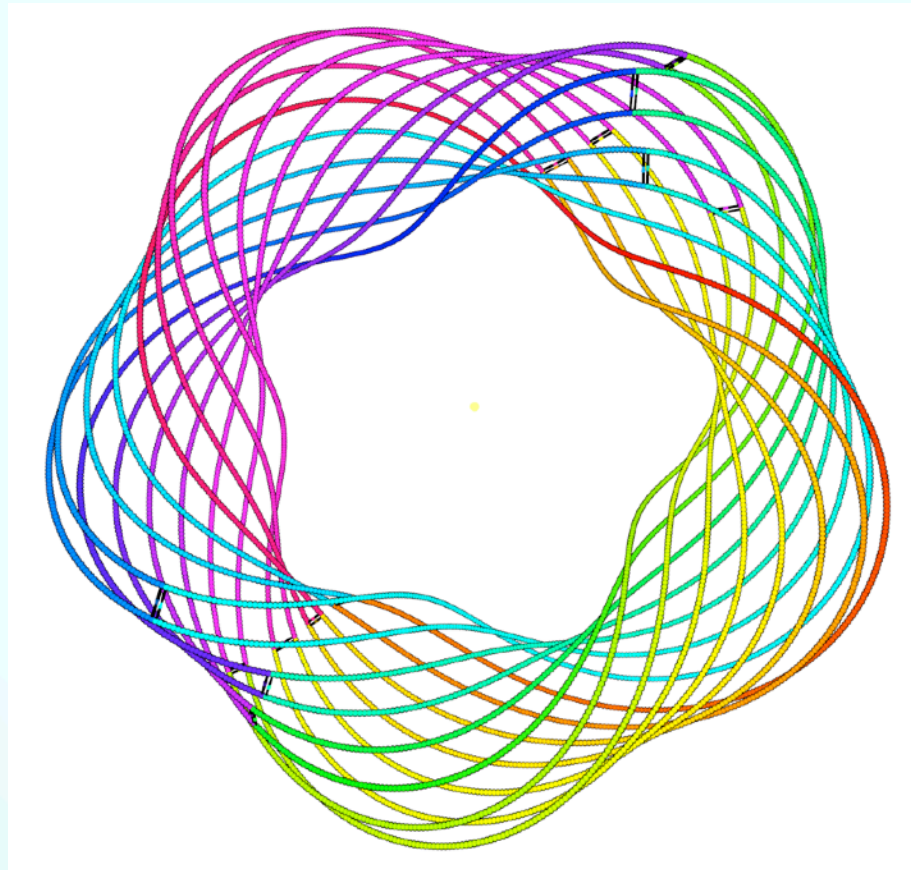
Folds poorly



Folds!



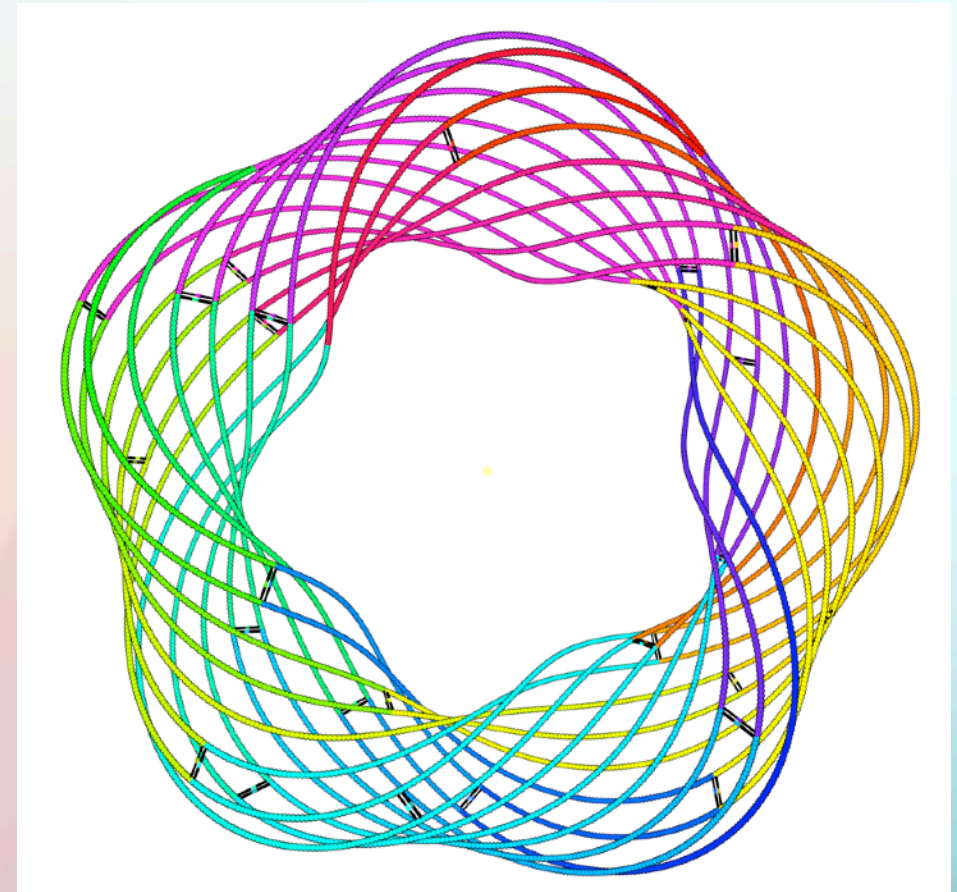
The scaffold route matters



Folds poorly



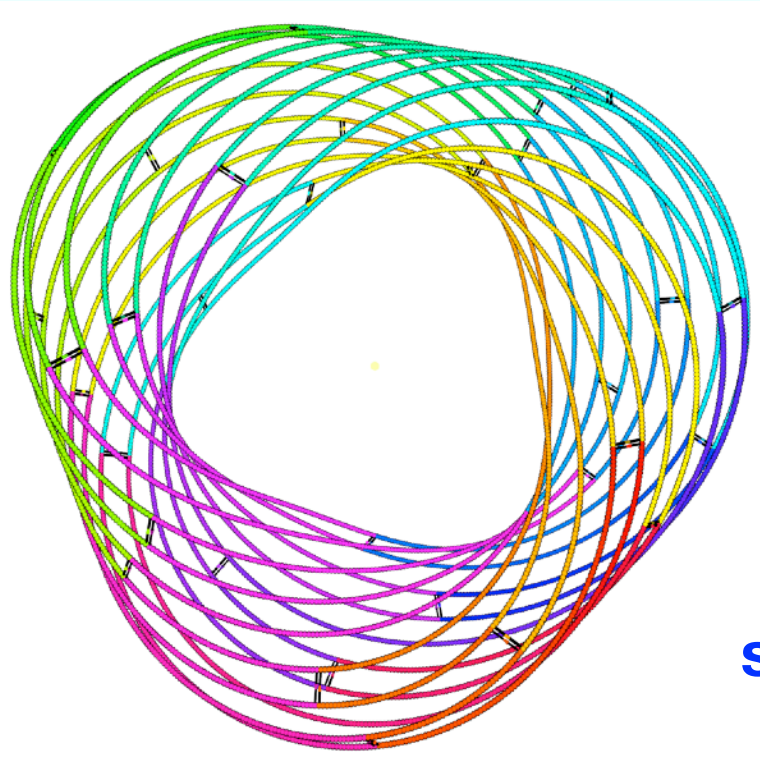
more cuts is better



Folds!



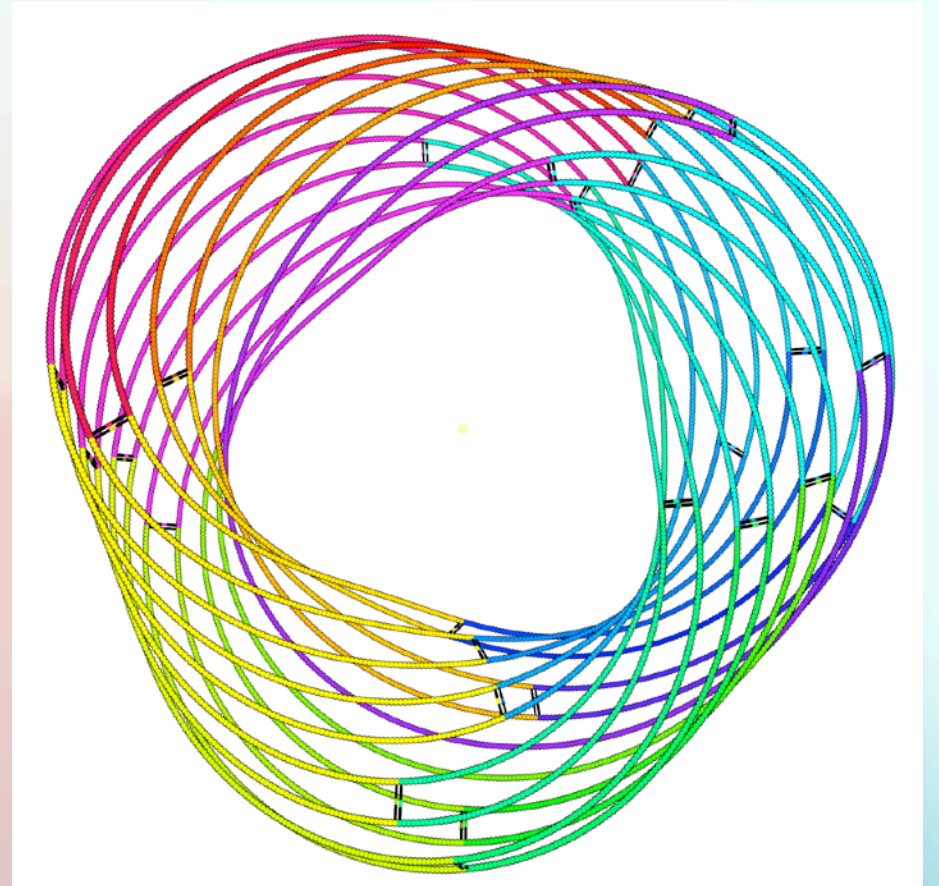
The scaffold route matters



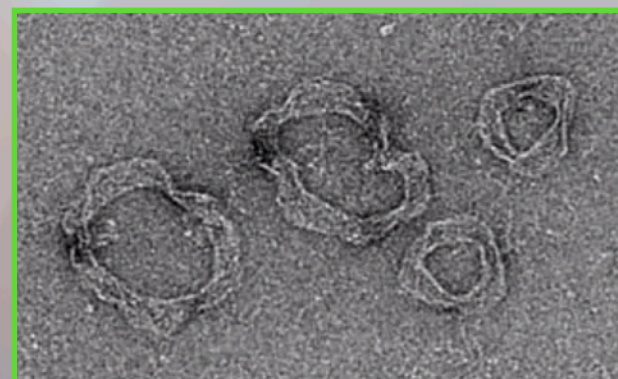
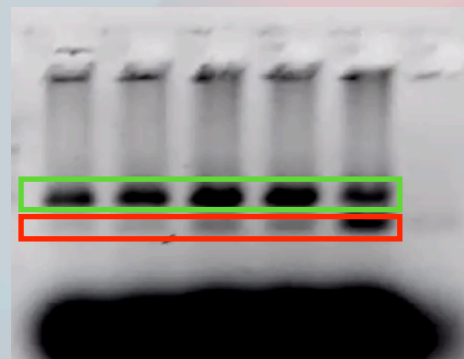
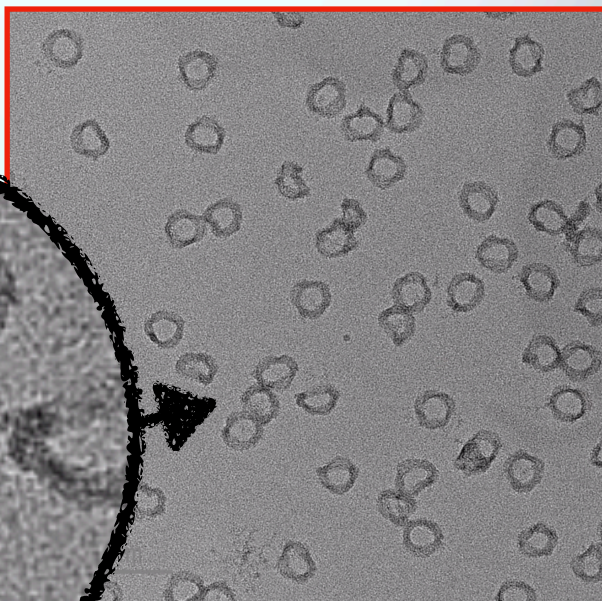
straight cuts help?



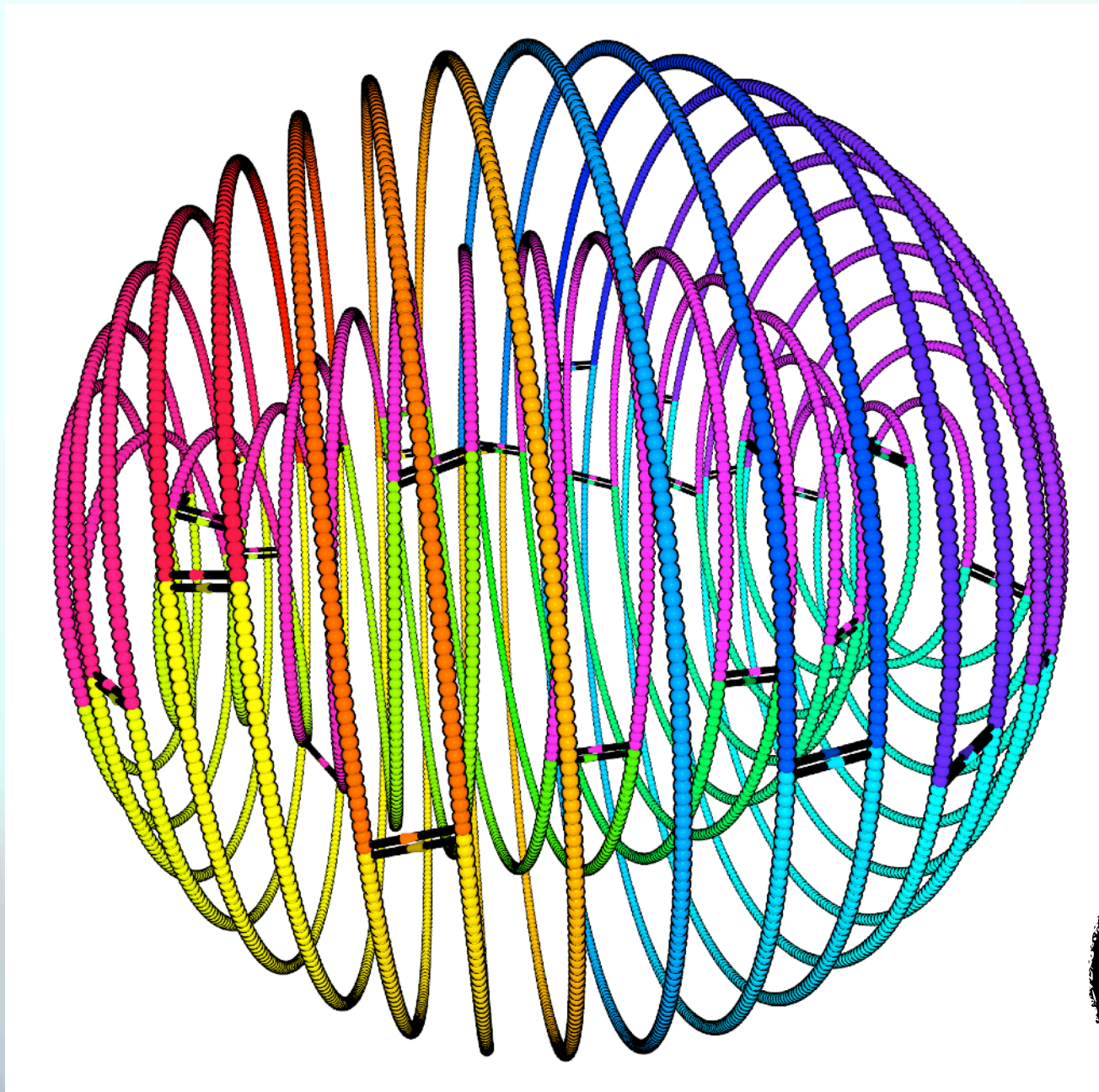
Folds poorly: makes chimera



Folds!

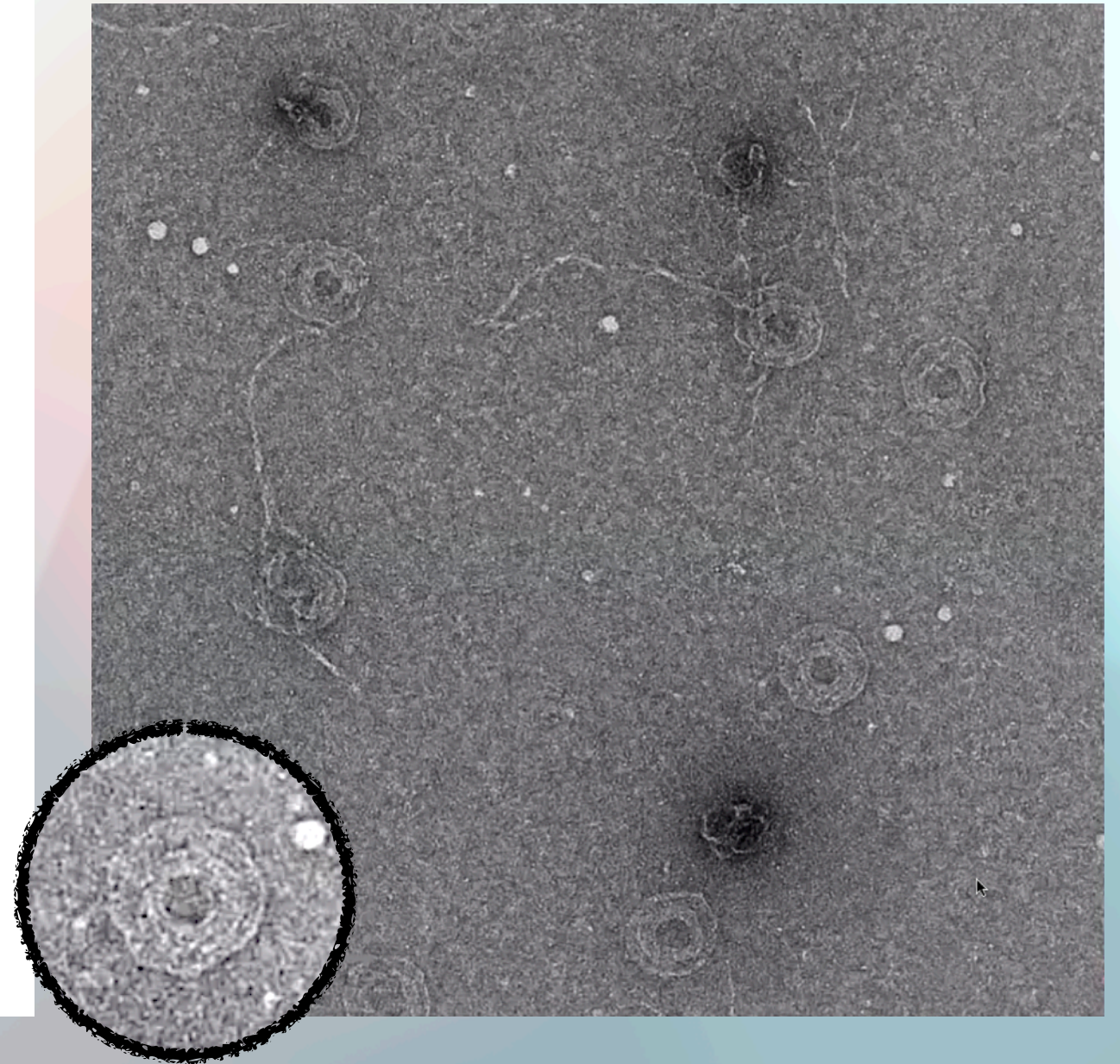


The scaffold route matters



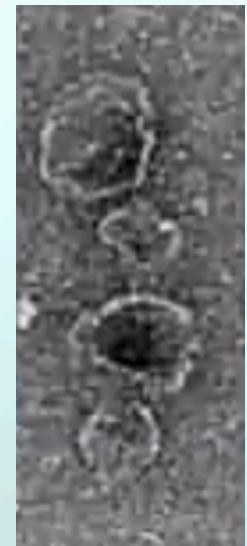
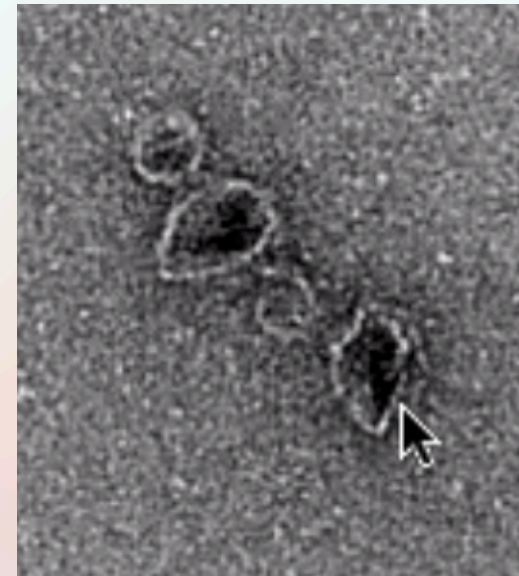
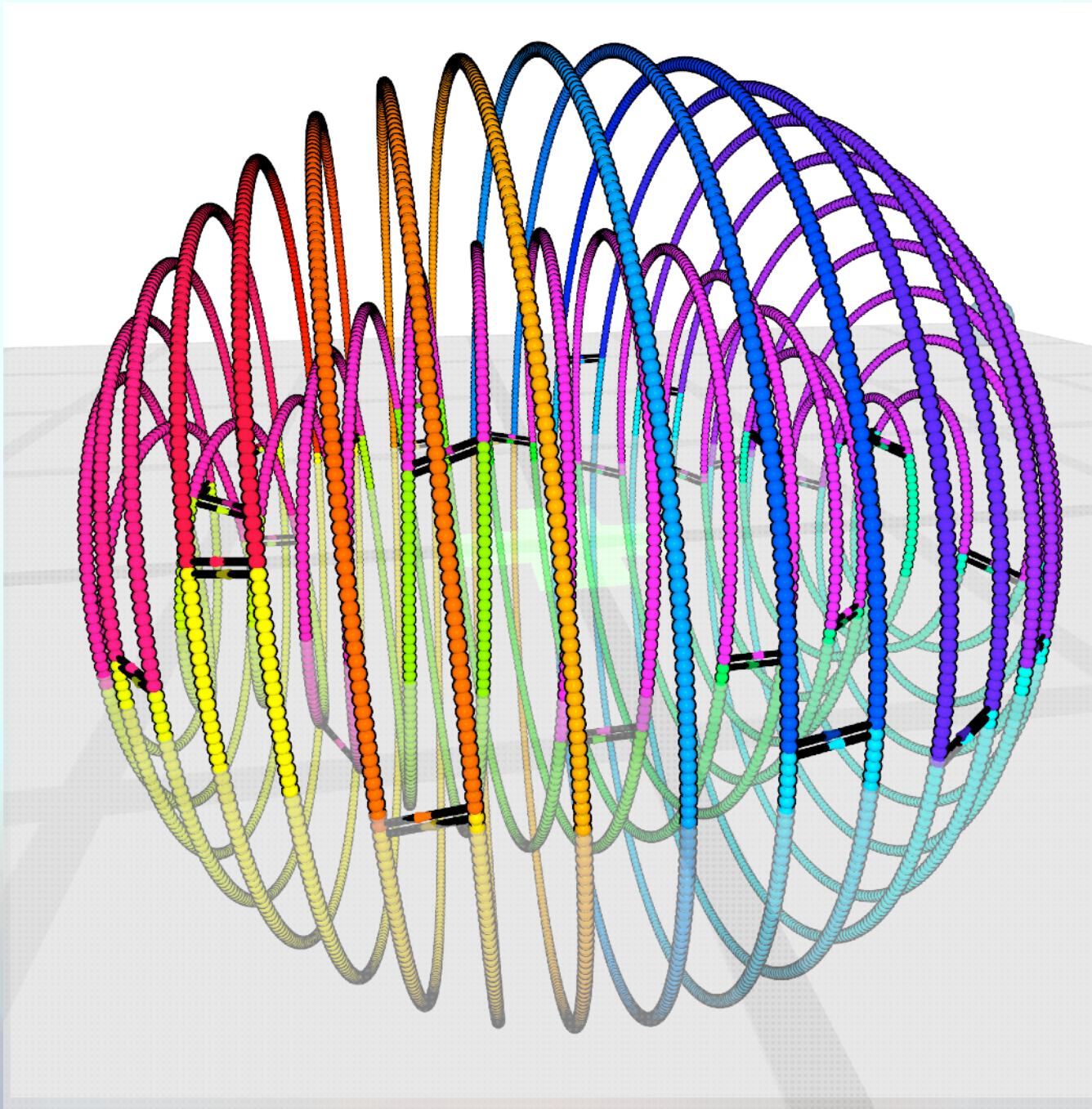
Death star 1: Folds!

cut in 2 is enough here



TEM by Gaëtan Bellot & Julie Finkel

The scaffold route matters



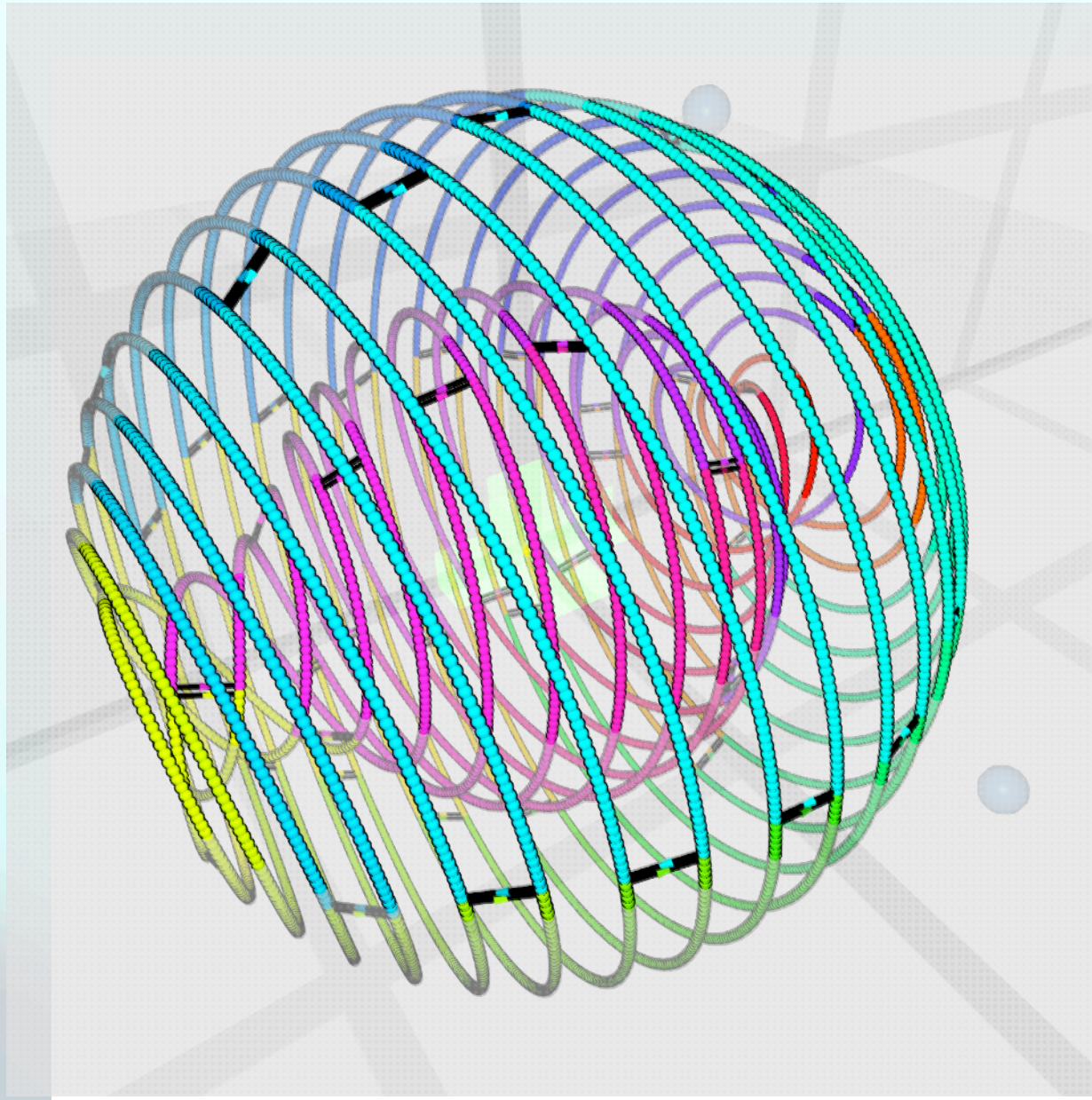
TEM by Gaëtan Bellot & Julie Finkel

Death star 2.1 (4 parallel helices): Fails to fold



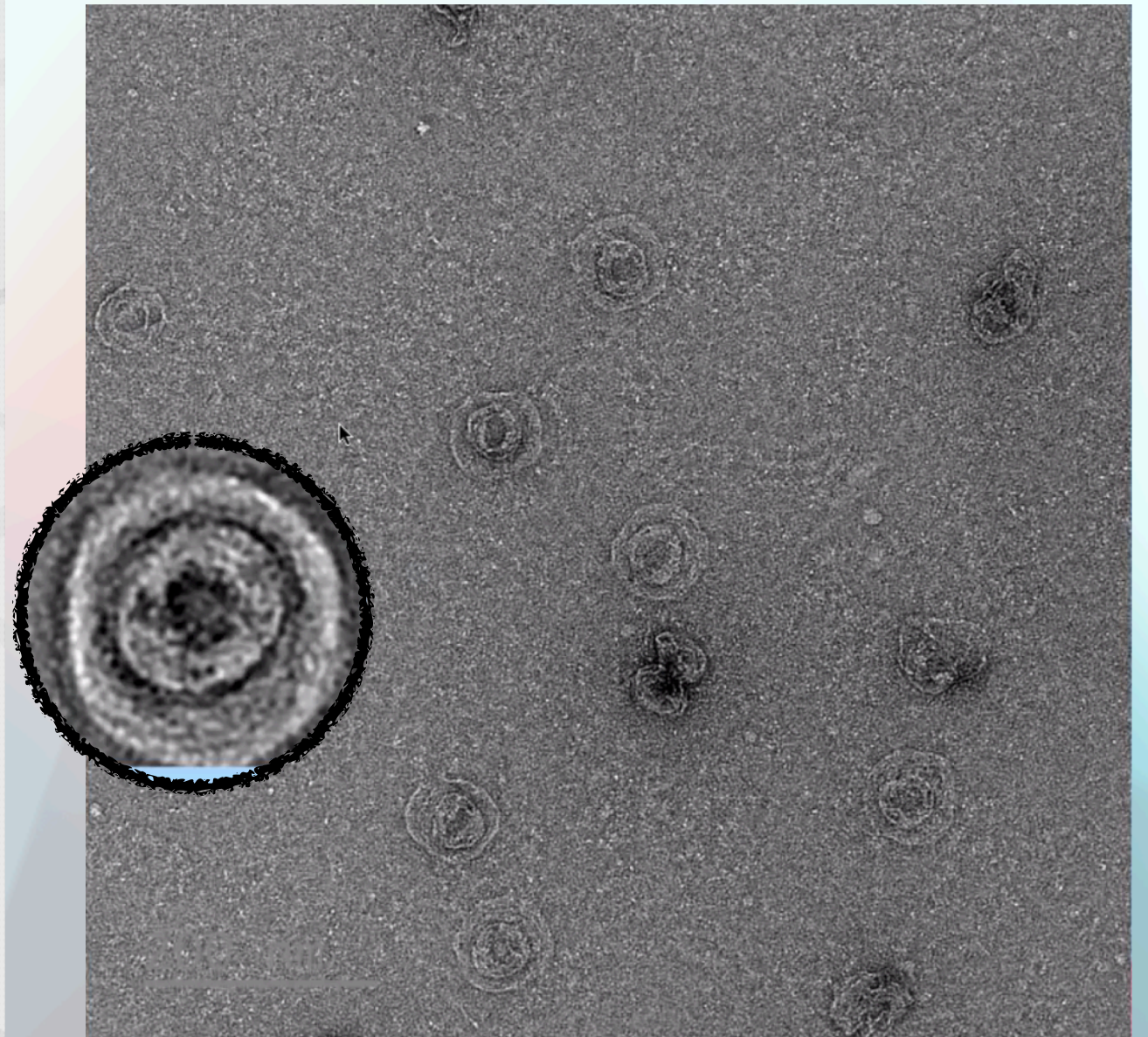
cut in 2 is NOT enough here

The scaffold route matters



Death star 2.2 (4 parallel helices): Folds!

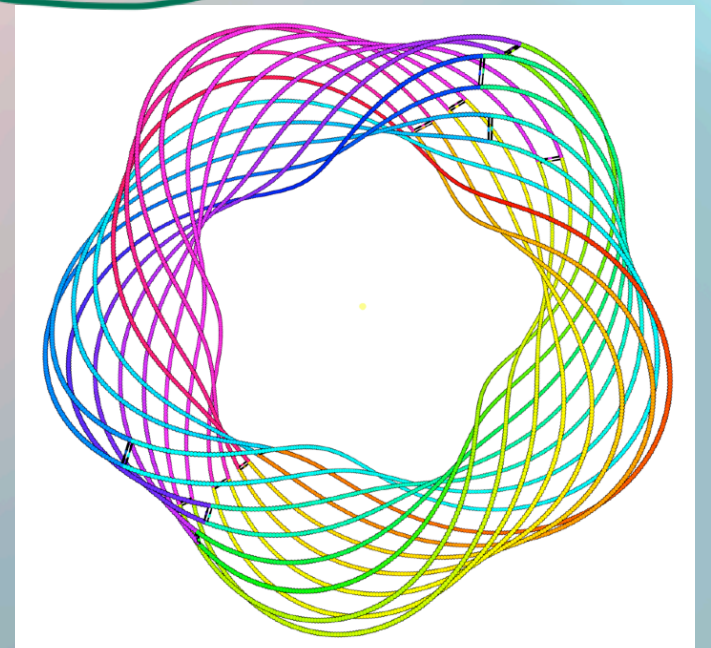
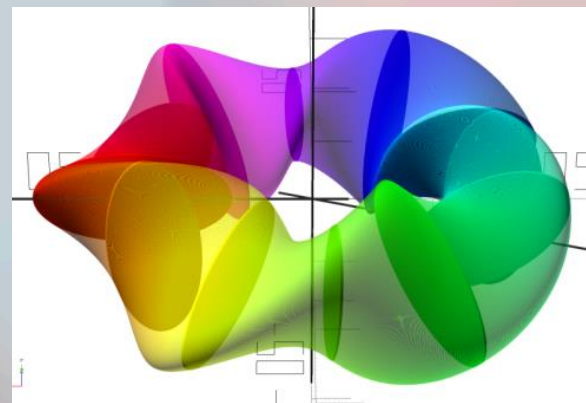
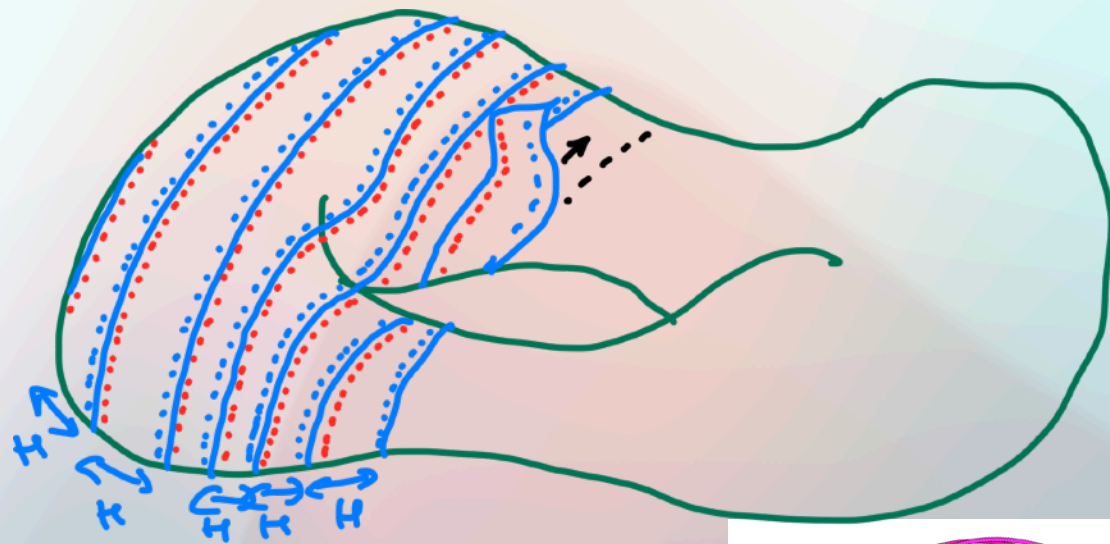
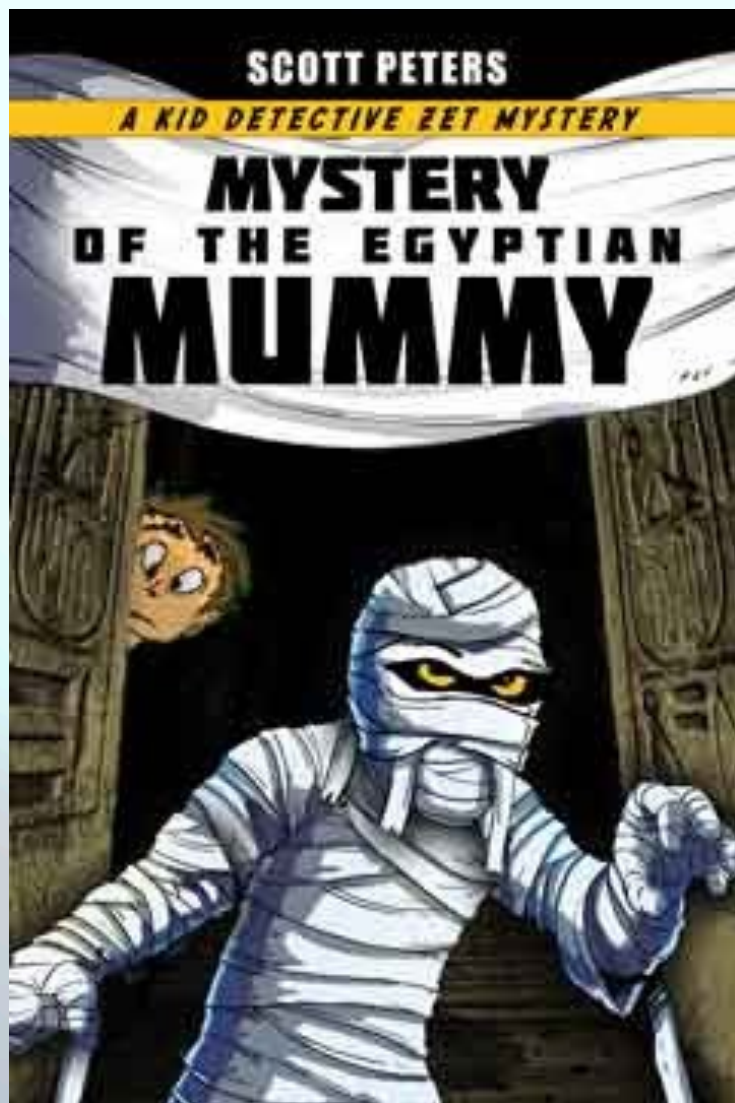
4 straight cuts of the outside sphere alone works here!



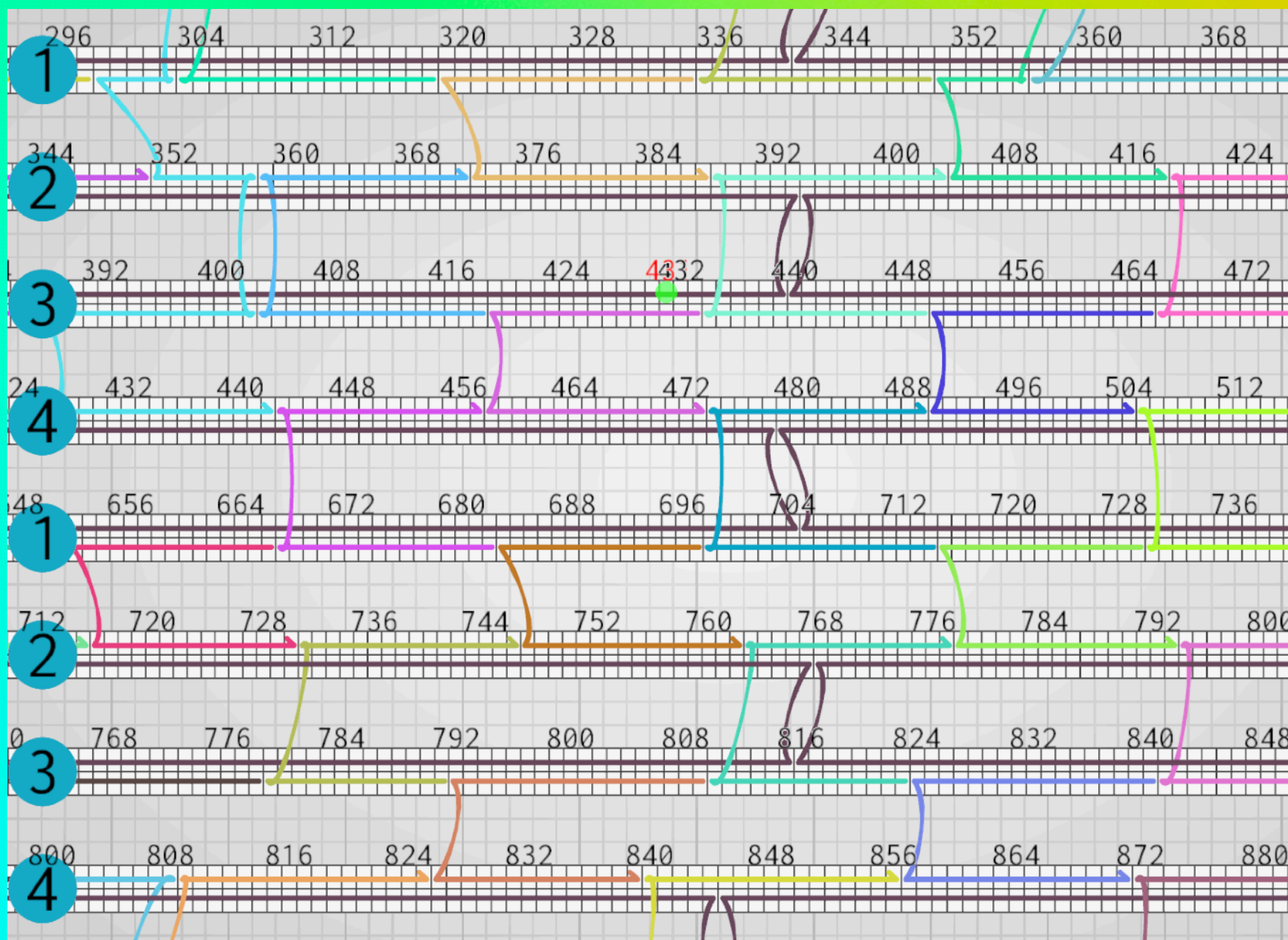
TEM by **Gaëtan Bellot & Julie Finkel**

The scaffold routing problem?

- Looking for an algorithmic formulation of the problem...



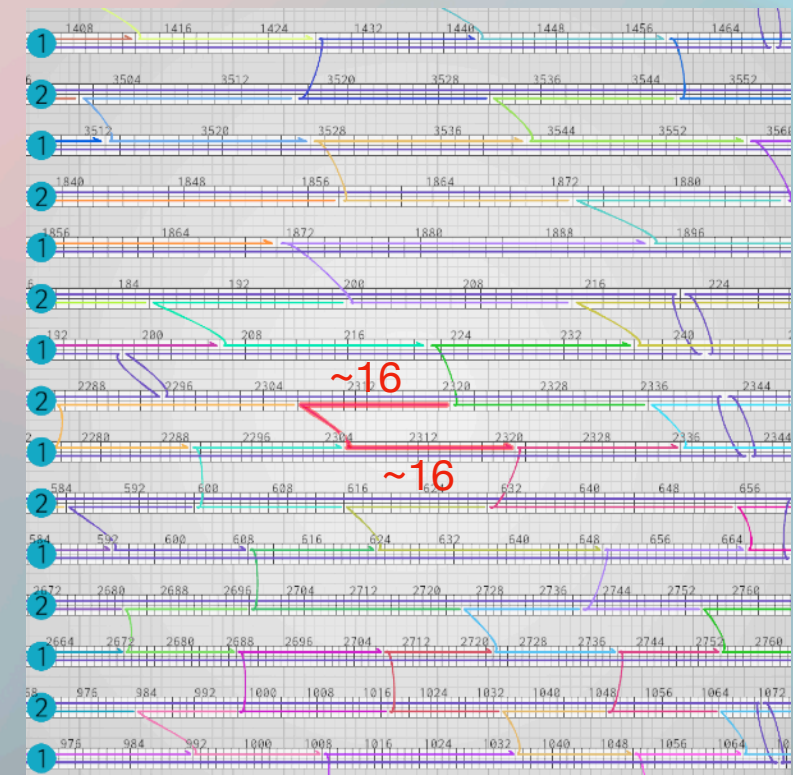
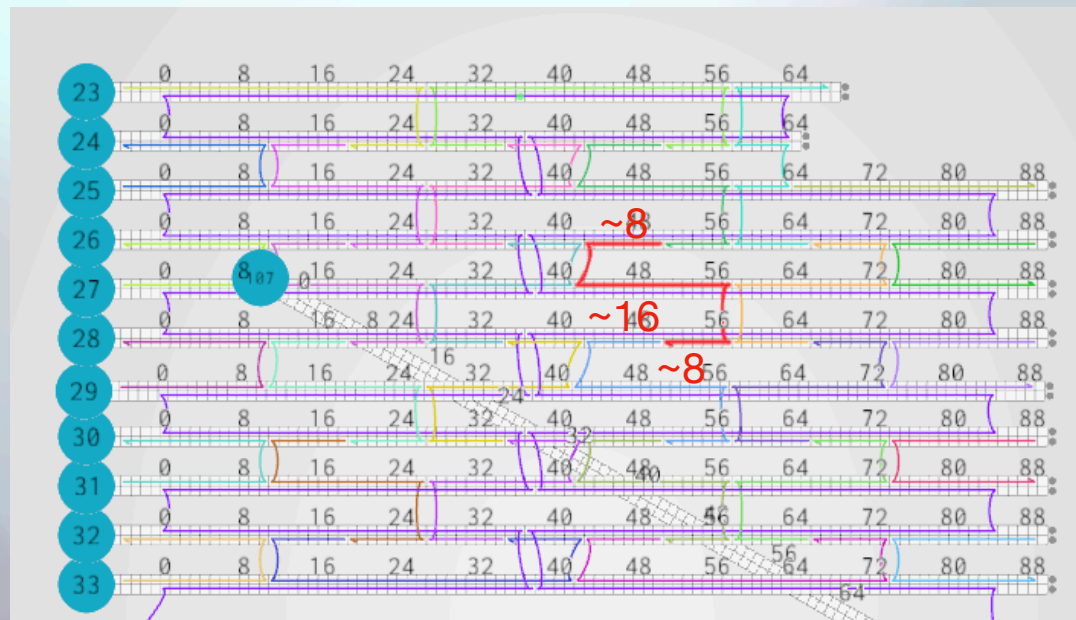
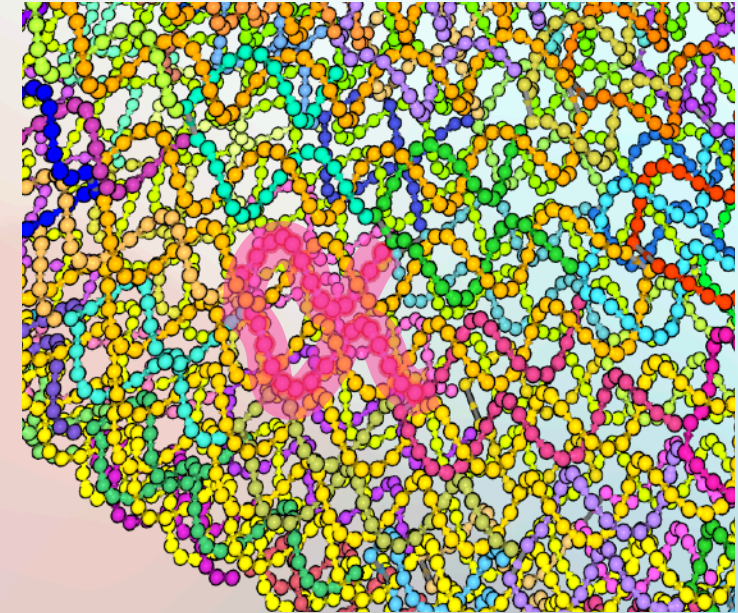
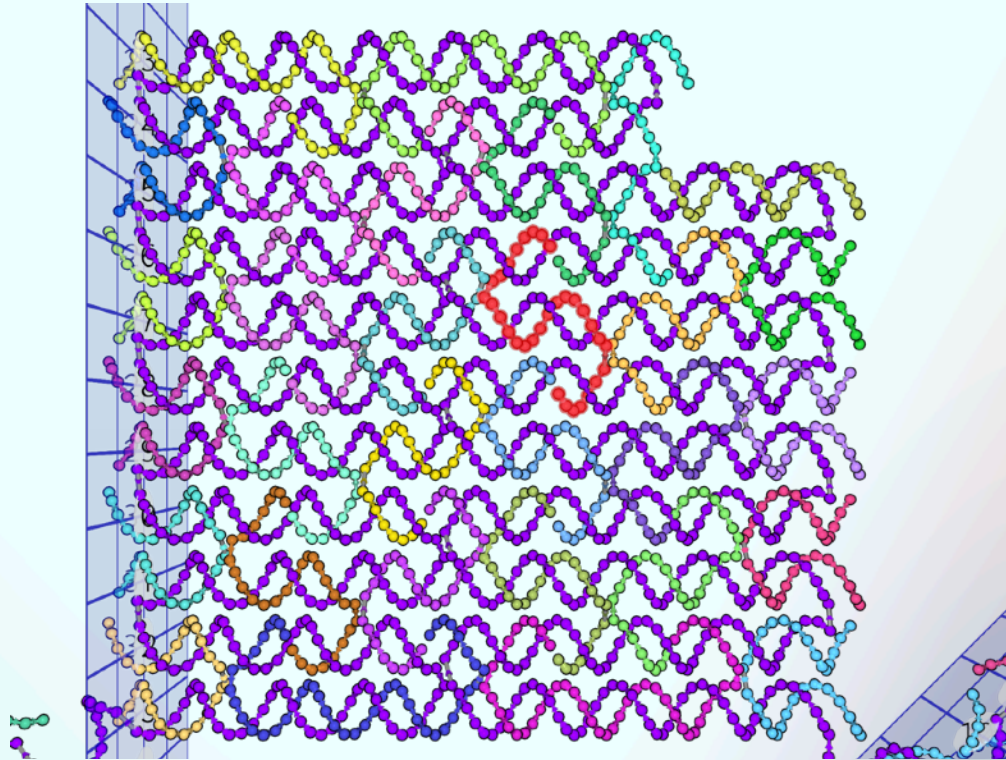
4. Stapling



Stapling

Staples are local and must tie the origami together

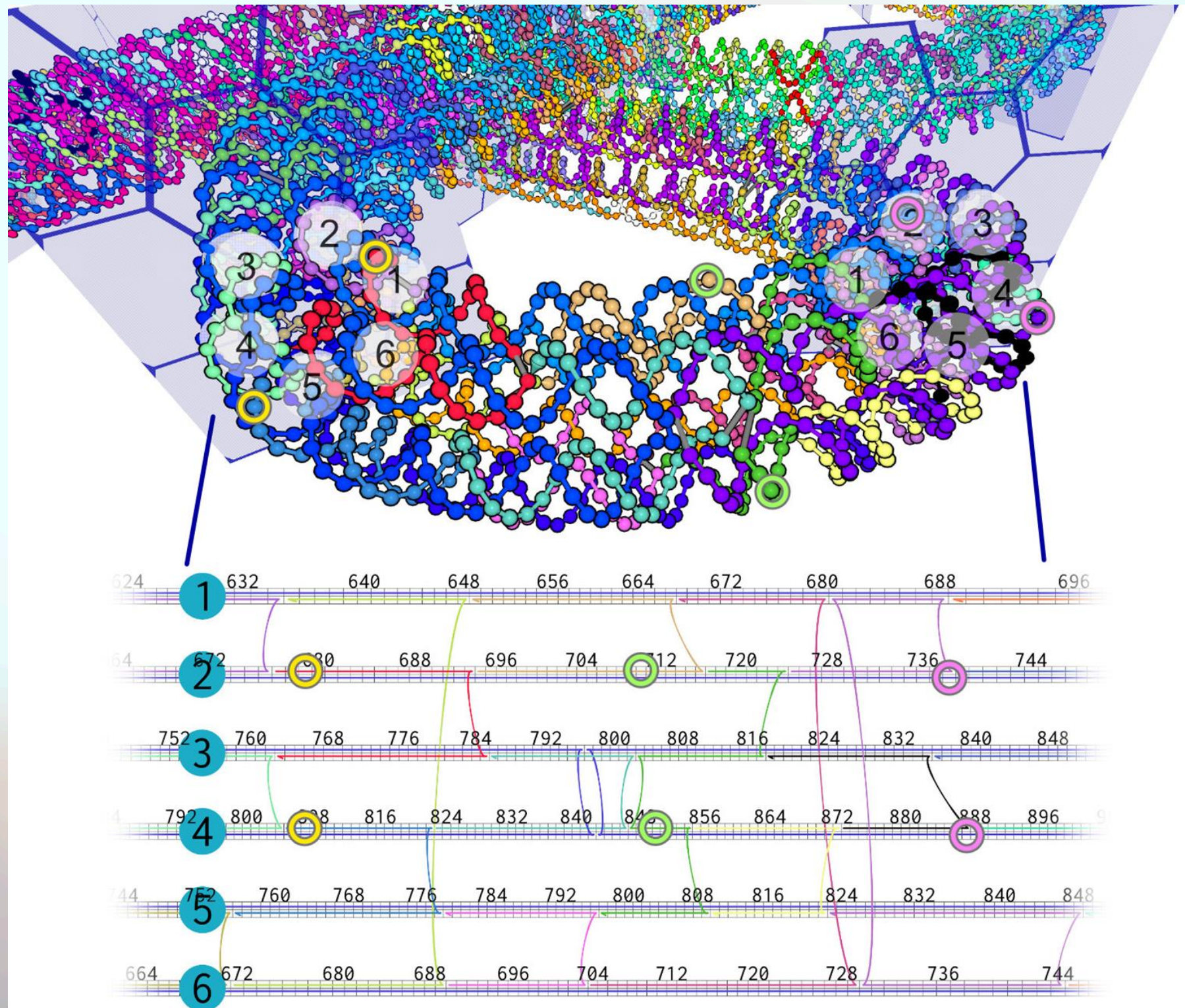
- Two main patterns for stapling 2D origamis: S and C



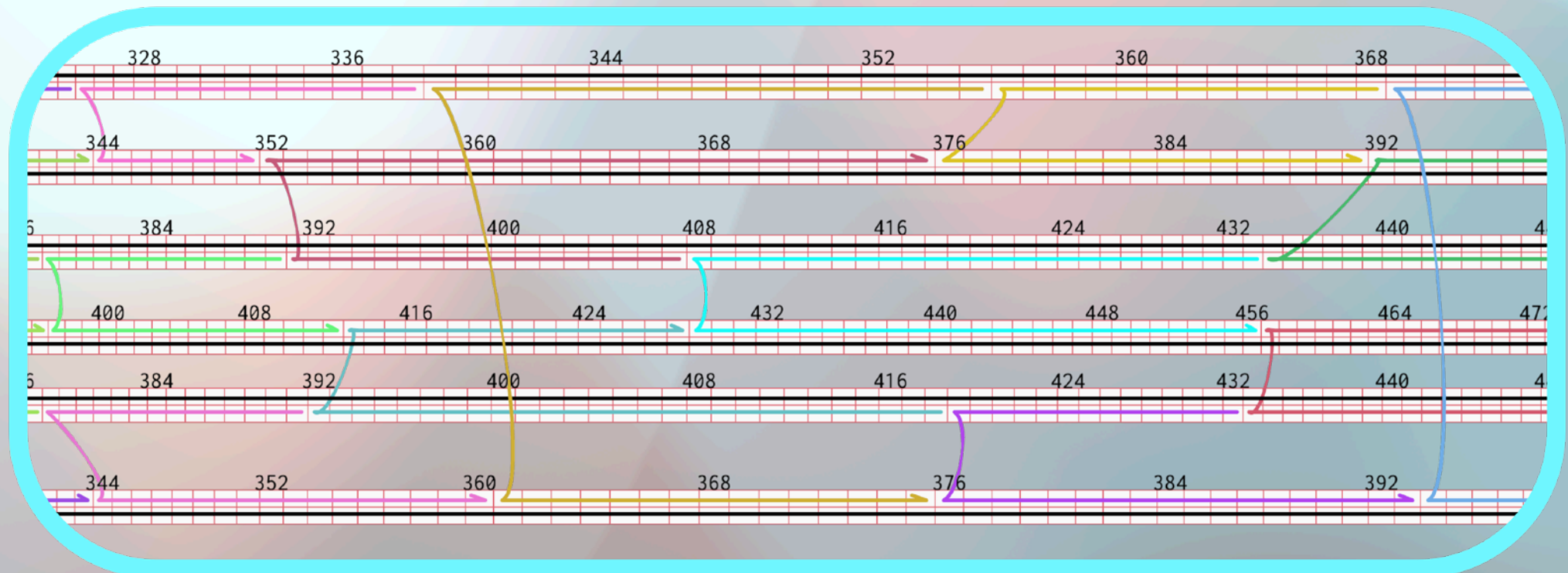
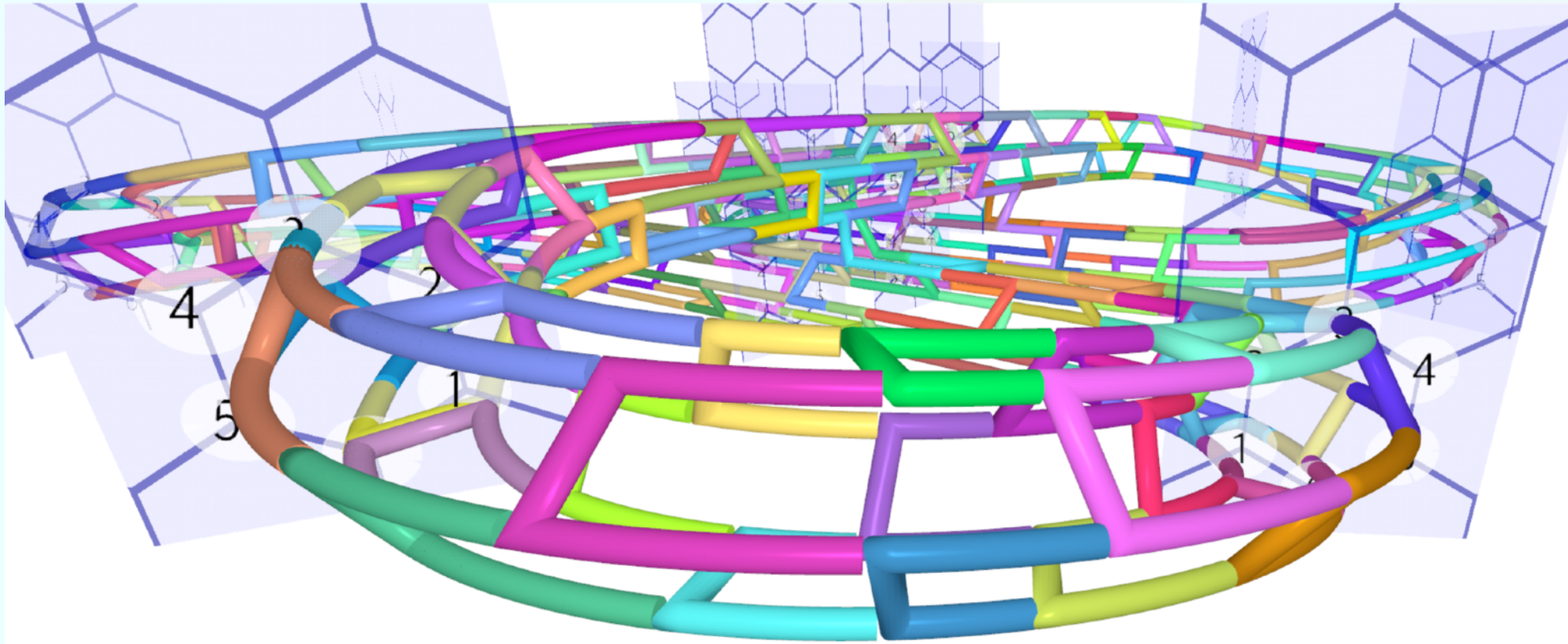
**Only few crosslinks are enough
even for curved shapes**



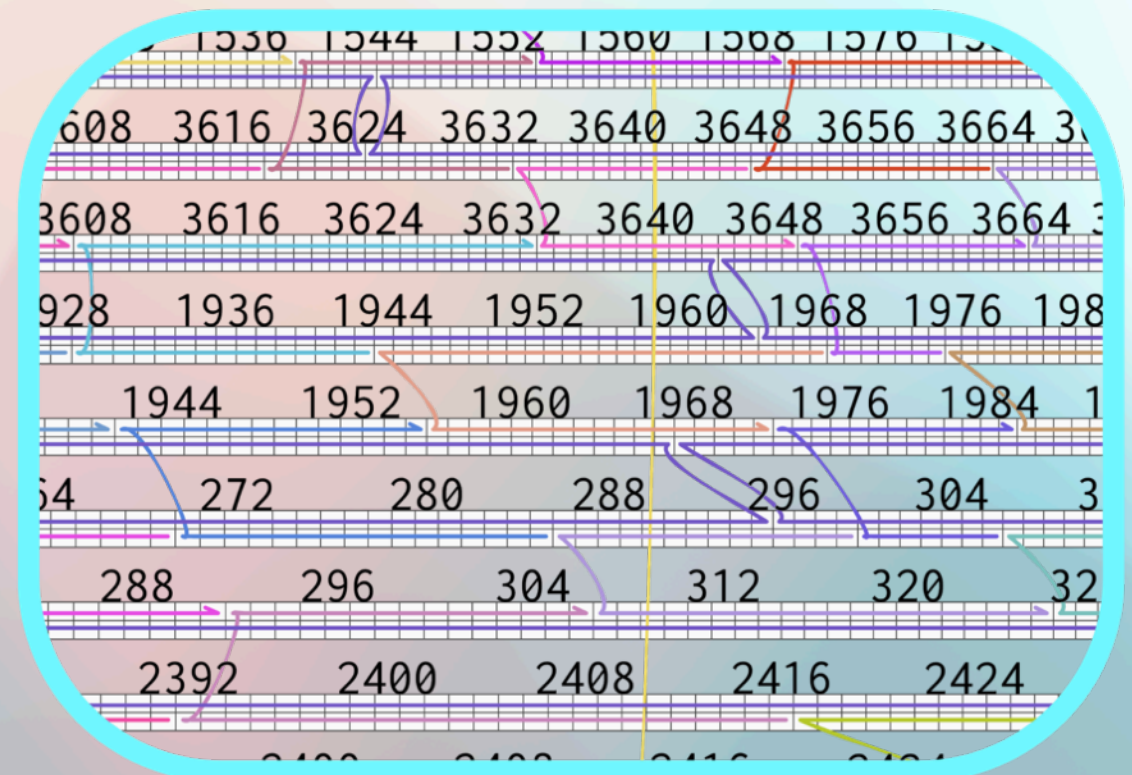
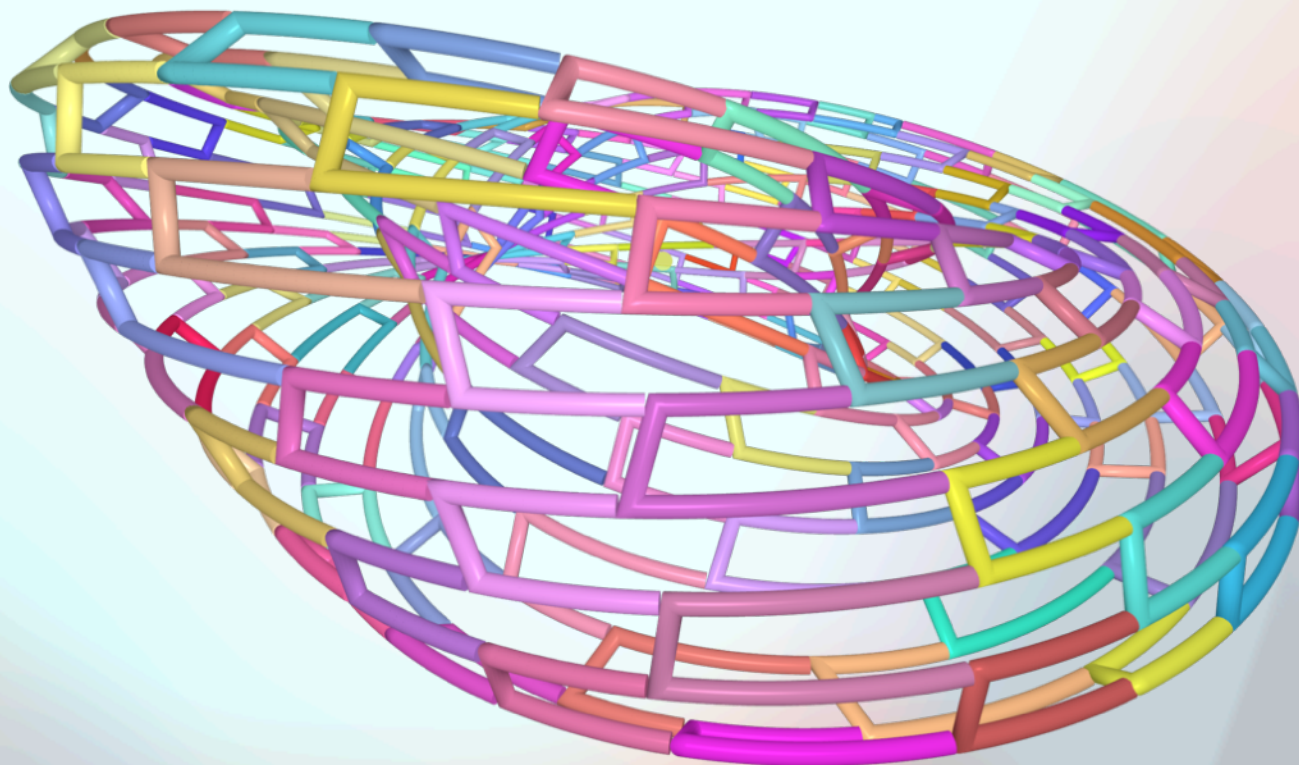
Stapling



Stapling

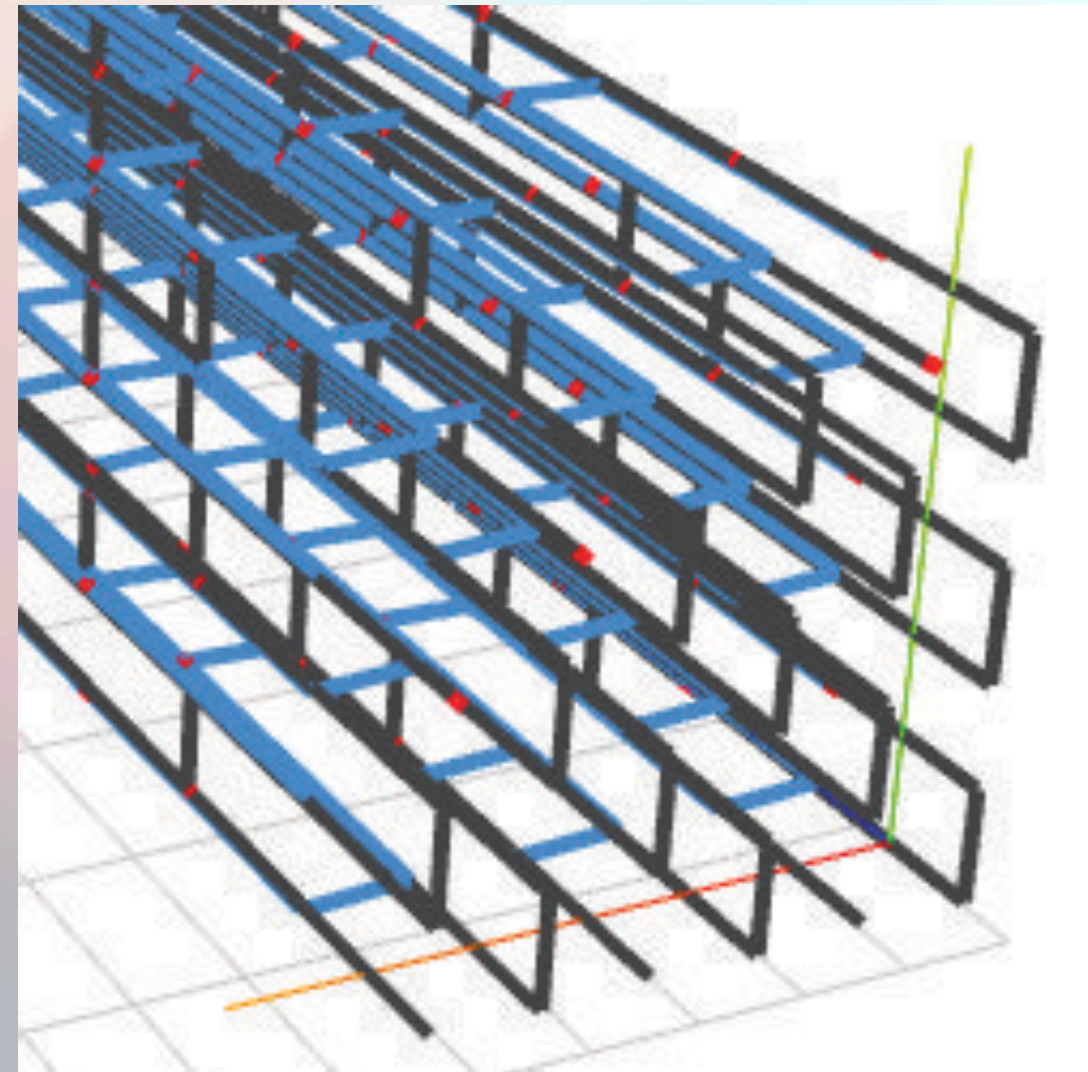
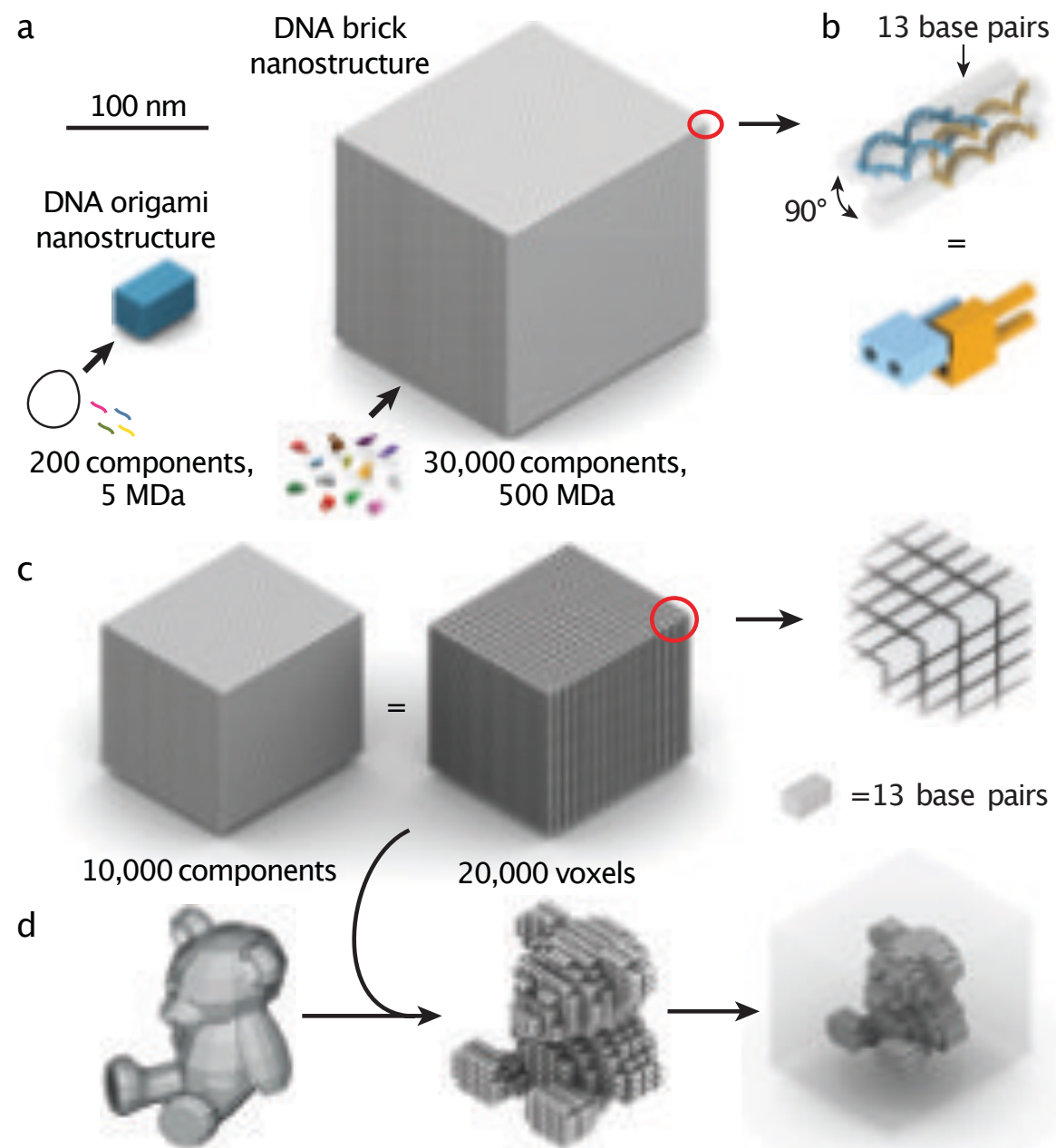


Stapling



Stapling

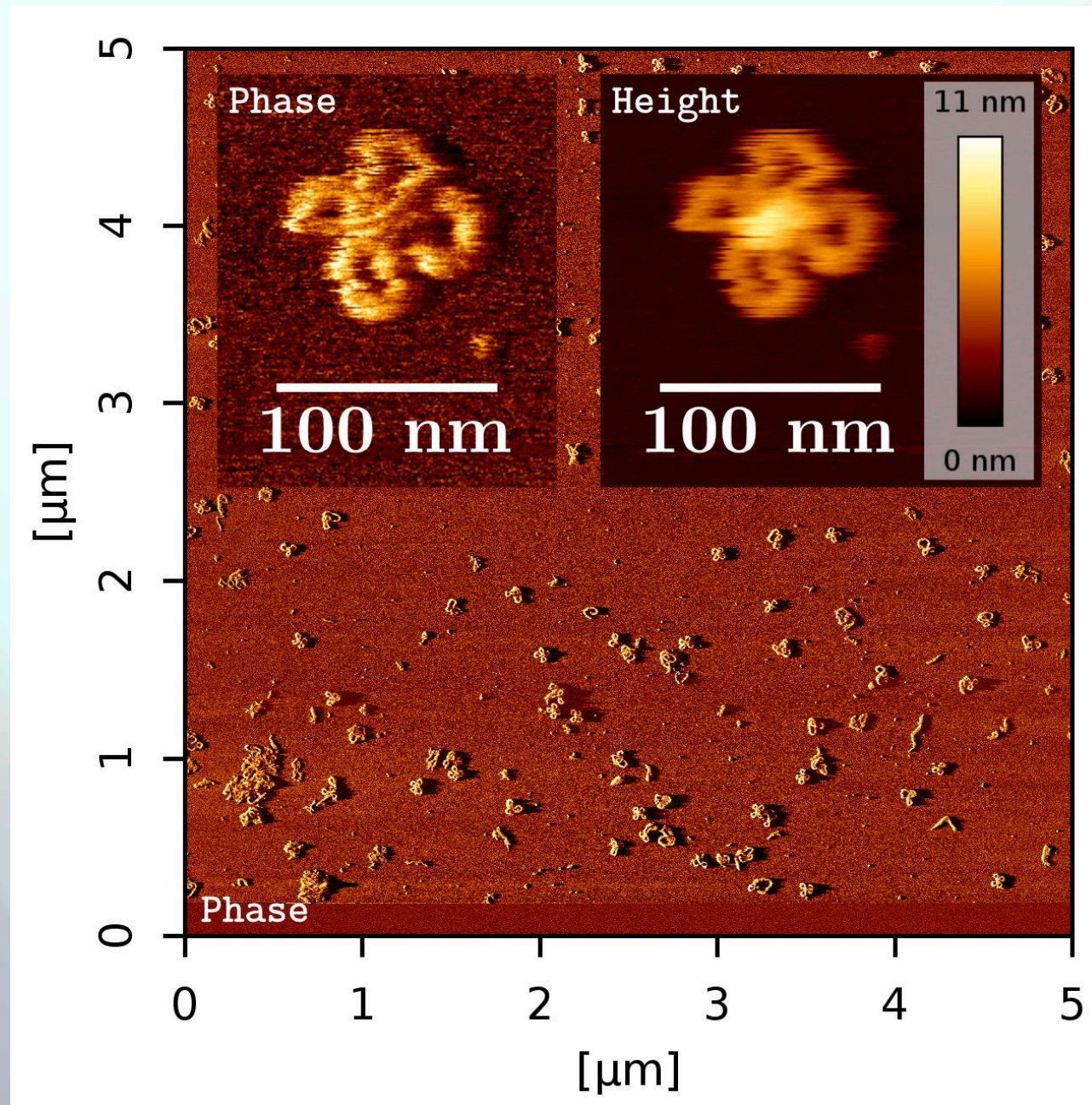
3D bundles



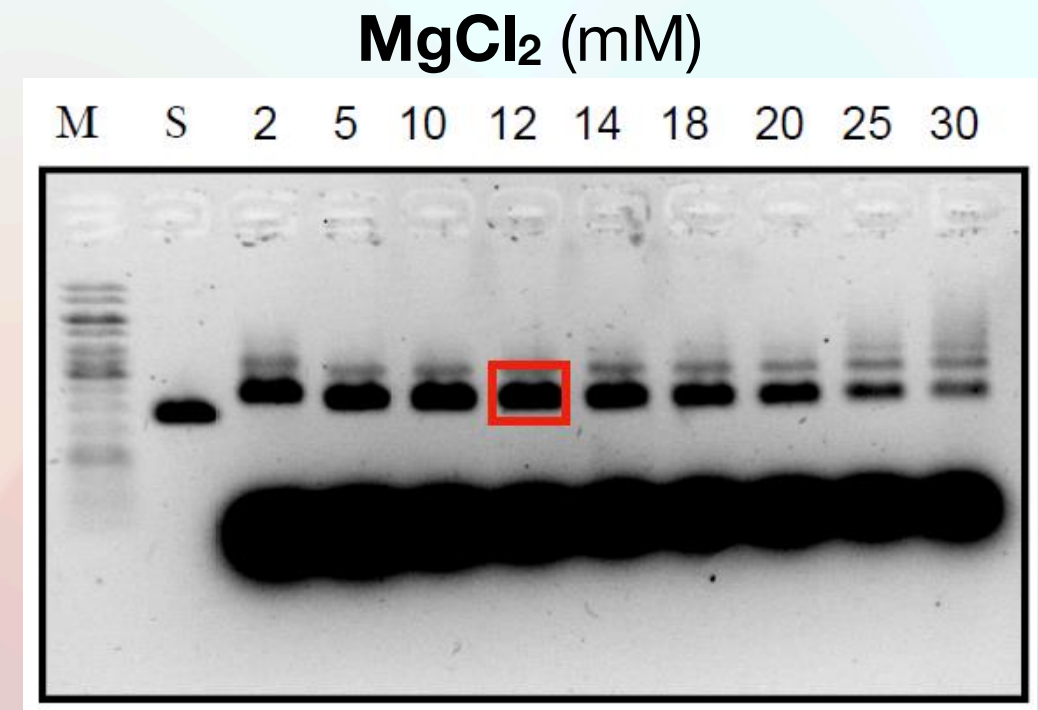
Experimental validation

Object	Denaturation phase (15 min) (°C)	Temperature ramp	Hold temperature (°C)	Folding ramp time period (h)	Magnesium chloride concentration (mM)	Scaffold	Scaffold concentration (nM)	Staples concentration (nM)
⌘-origami	65	60°C to 40°C: -1°C/2h	10	42	20	M13 7249	20	200
Tore 1	65	60°C to 40°C: -1°C/2h	10	42	12	M13 7560	20	200
Tore 2	65	1) 60°C to 55°C: -1°C/10min 2) 55°C to 25°C: -1°C/1h	25	33	18	M13 7560	20	200
Tore 3	65	1) 60°C to 55°C: -1°C/10min 2) 55°C to 25°C: -1°C/1h	25	33	18	M13 7560	20	200
Tore 4	65	1) 60°C to 55°C: -1°C/10min 2) 55°C to 25°C: -1°C/1h	25	33	18	M13 7560	20	200
Tore 5	65	60°C to 40°C: -1°C/10min	10	3.5	18	M13 7560	5	50
Tore 6	65	1) 60°C to 55°C: -1°C/10min 2) 55°C to 25°C: -1°C/1h	25	33	18	M13 7560	20	200
DS1	65	60°C to 40°C: -1°C/10min	10	3.5	18	M13 8064	20	200
DS2	65	60°C to 40°C: -1°C/10min	10	3.5	16	M13 8064	20	200
Vault	65	60°C to 40°C: -1°C/3h	10	63	20	M13 8064	20	200

Bezier curves

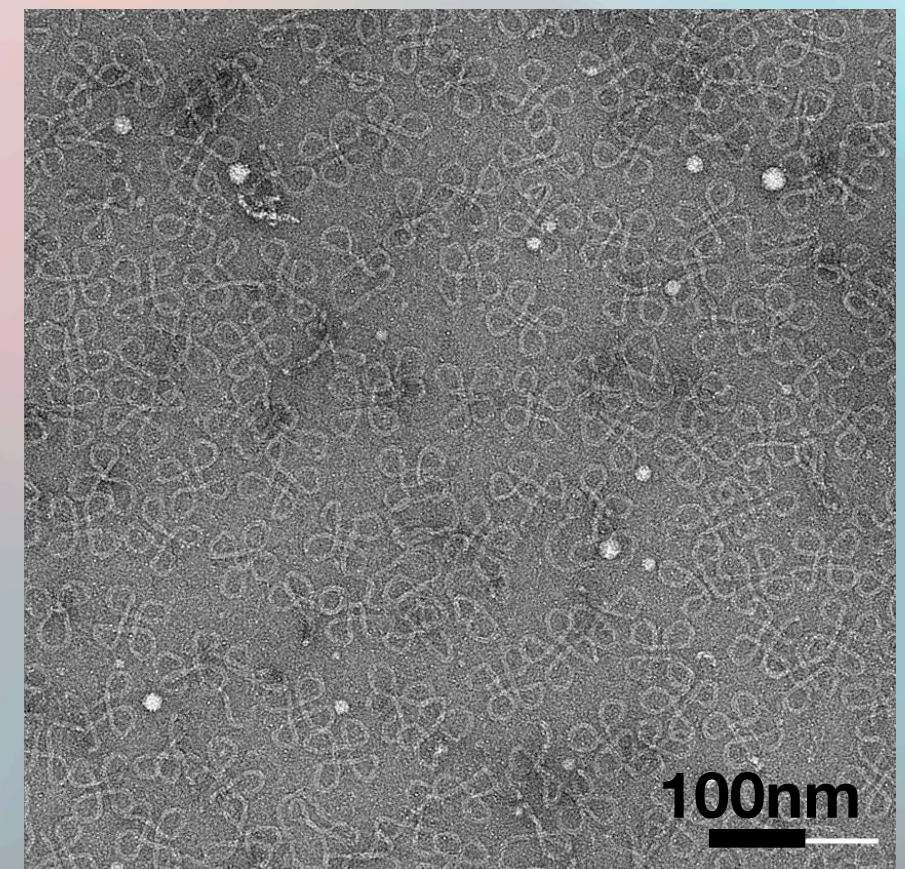


Unpurified



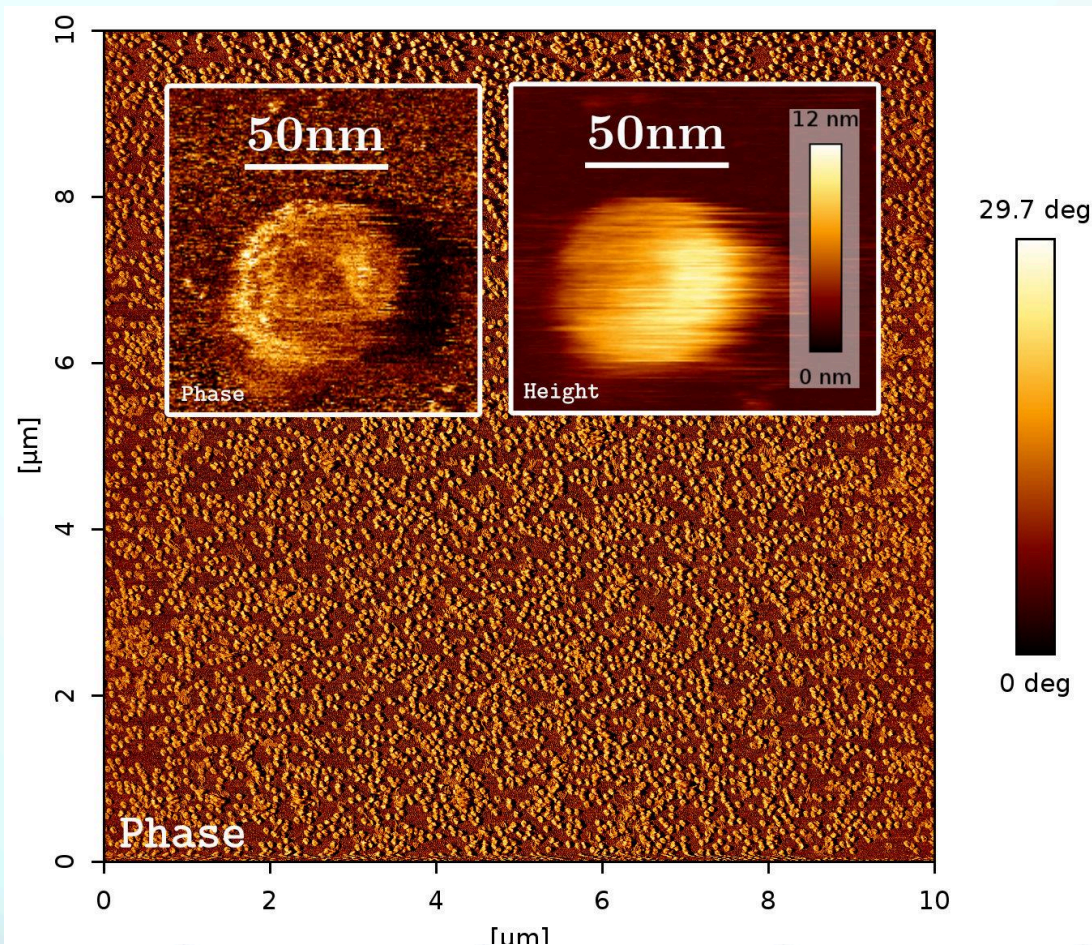
TEM by
G. Bellot,
J. Finkel

Purified



Twisted revolution surfaces

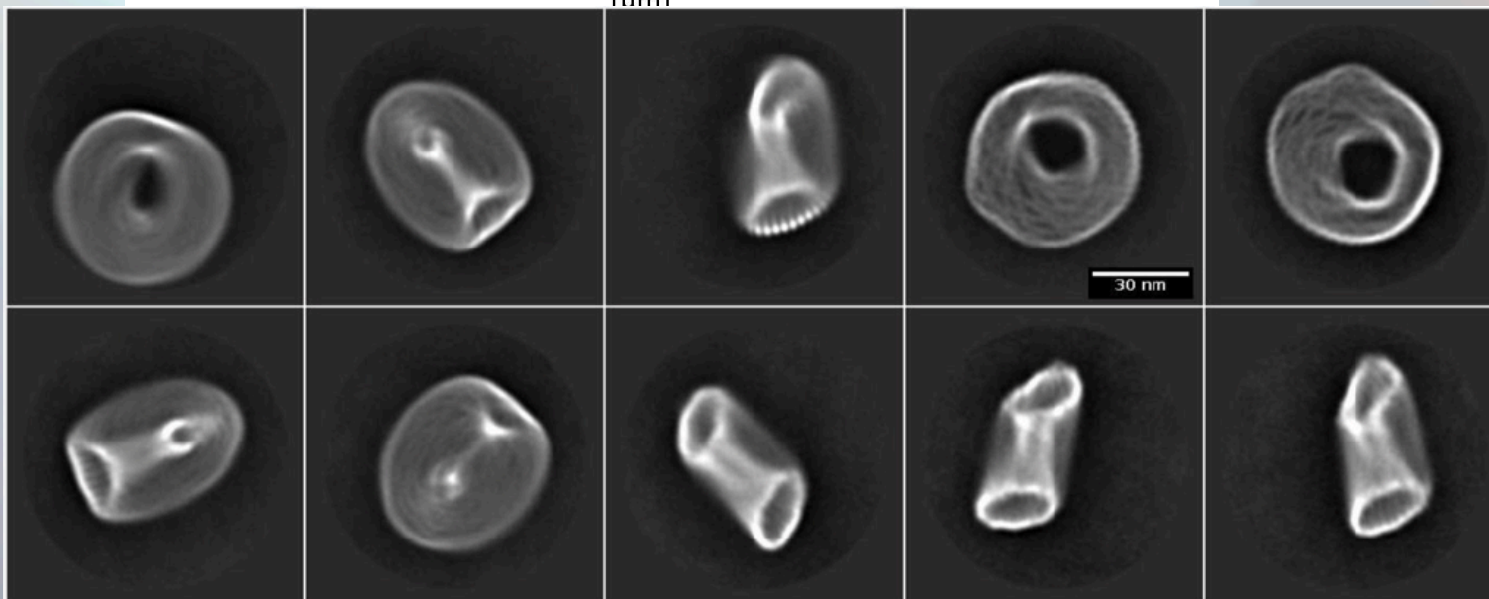
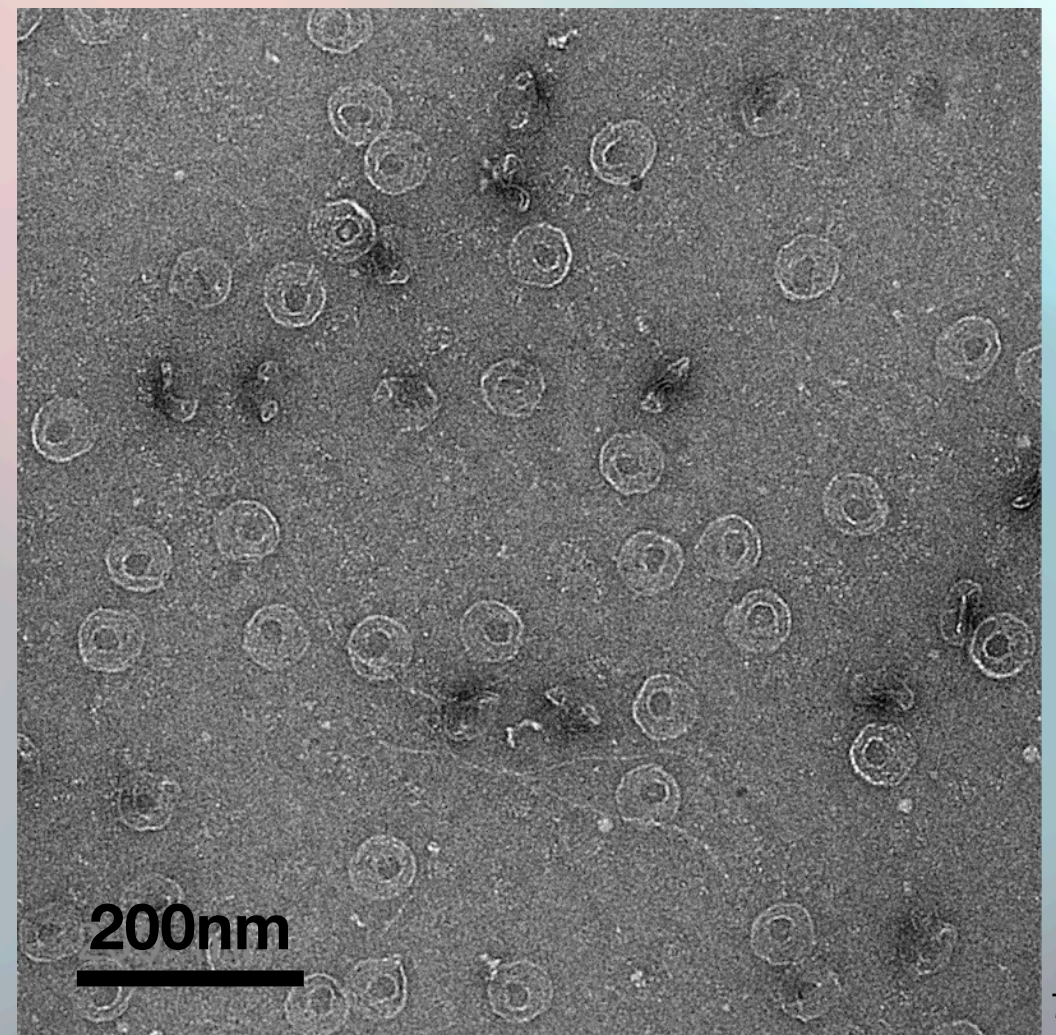
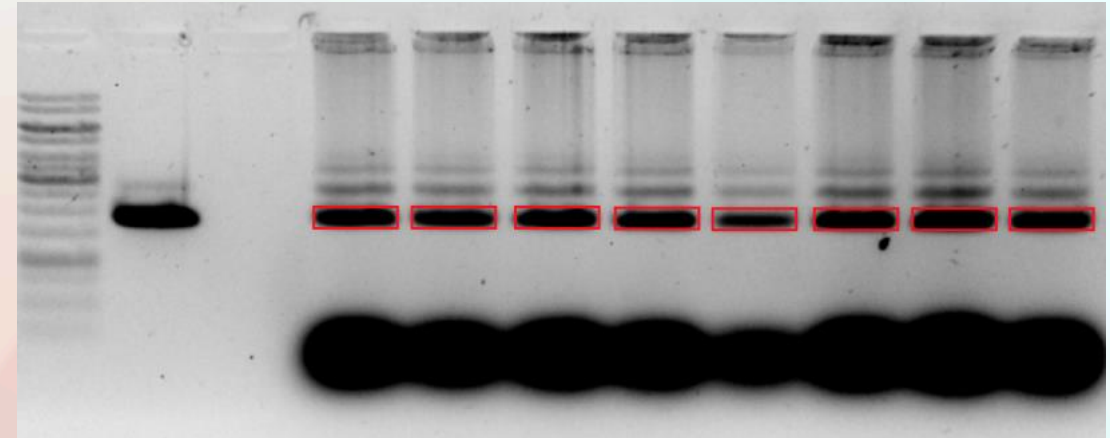
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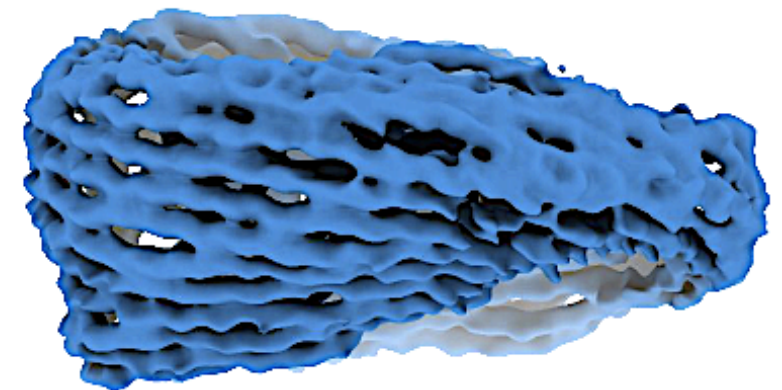
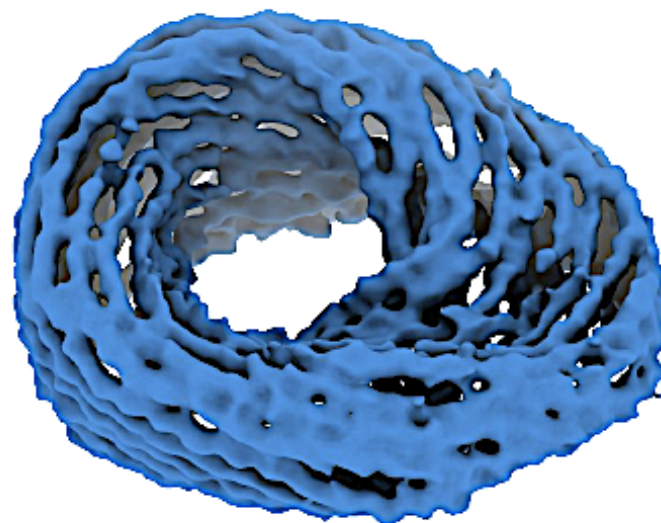
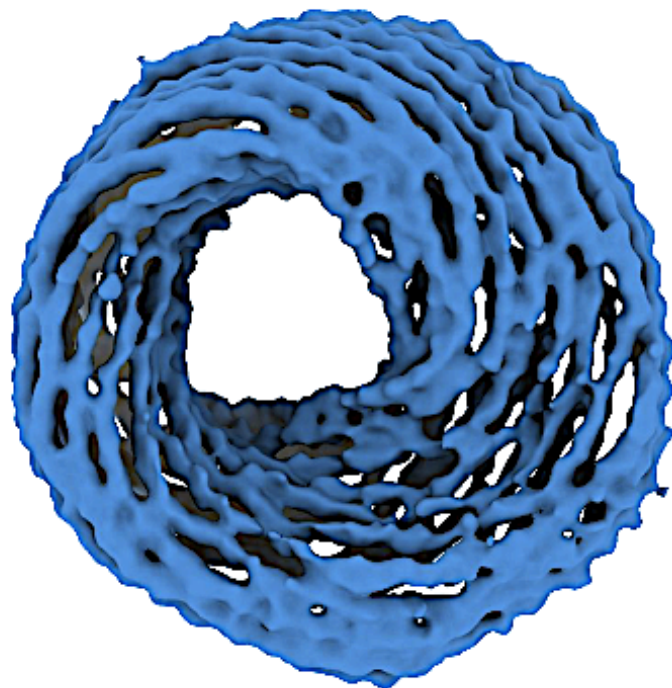
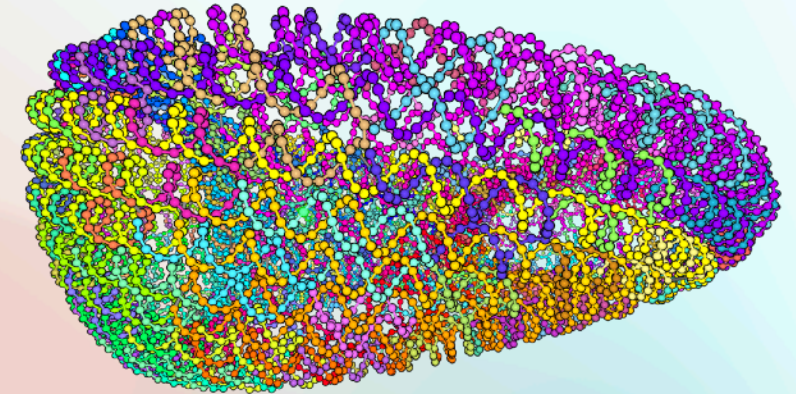
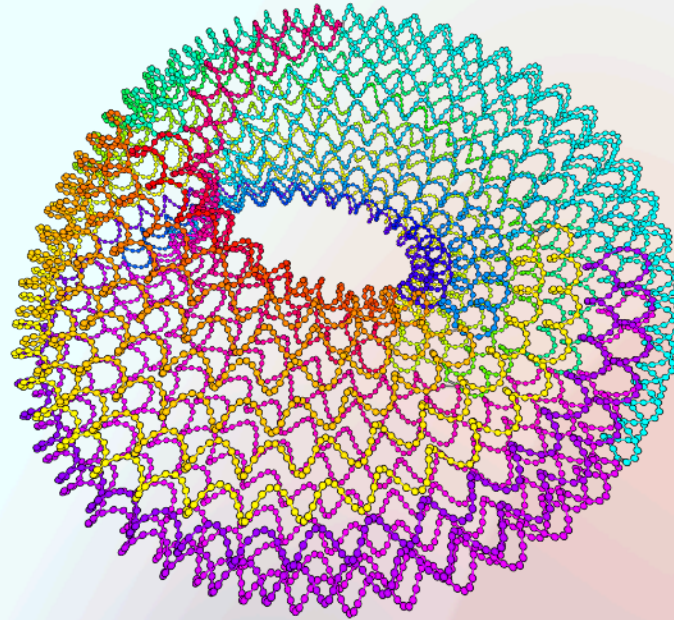
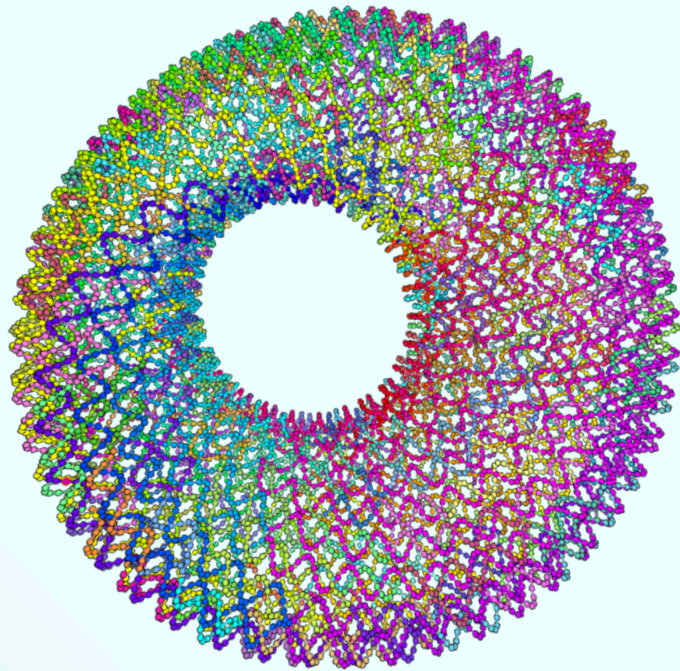
TEM by
G. Bellot,
J. Finkel

Purified

MgCl₂ (12 mM)



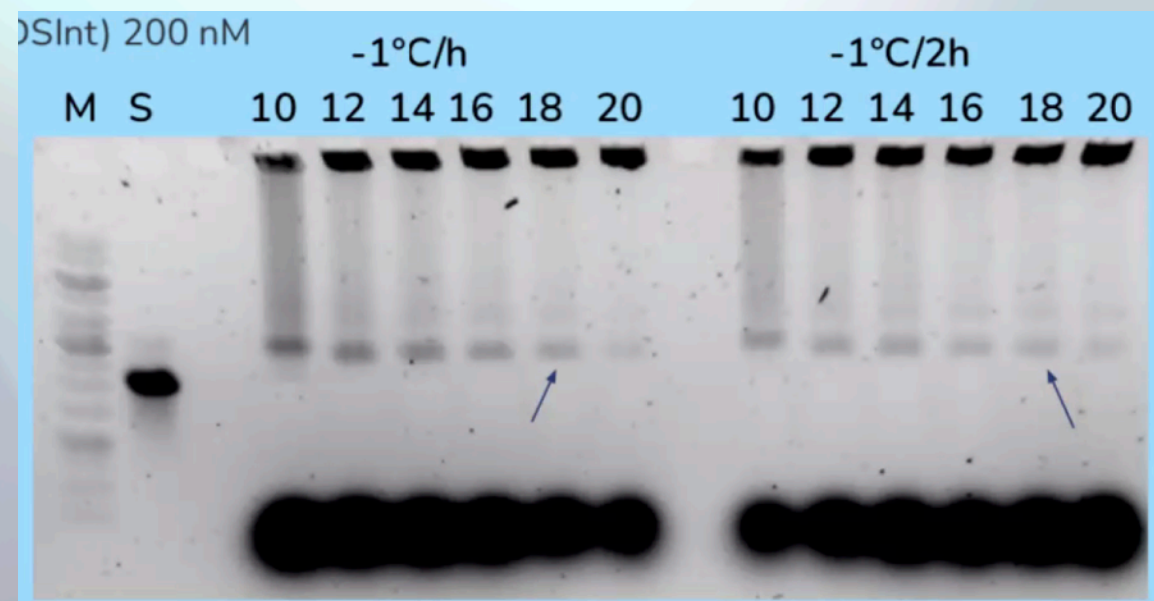
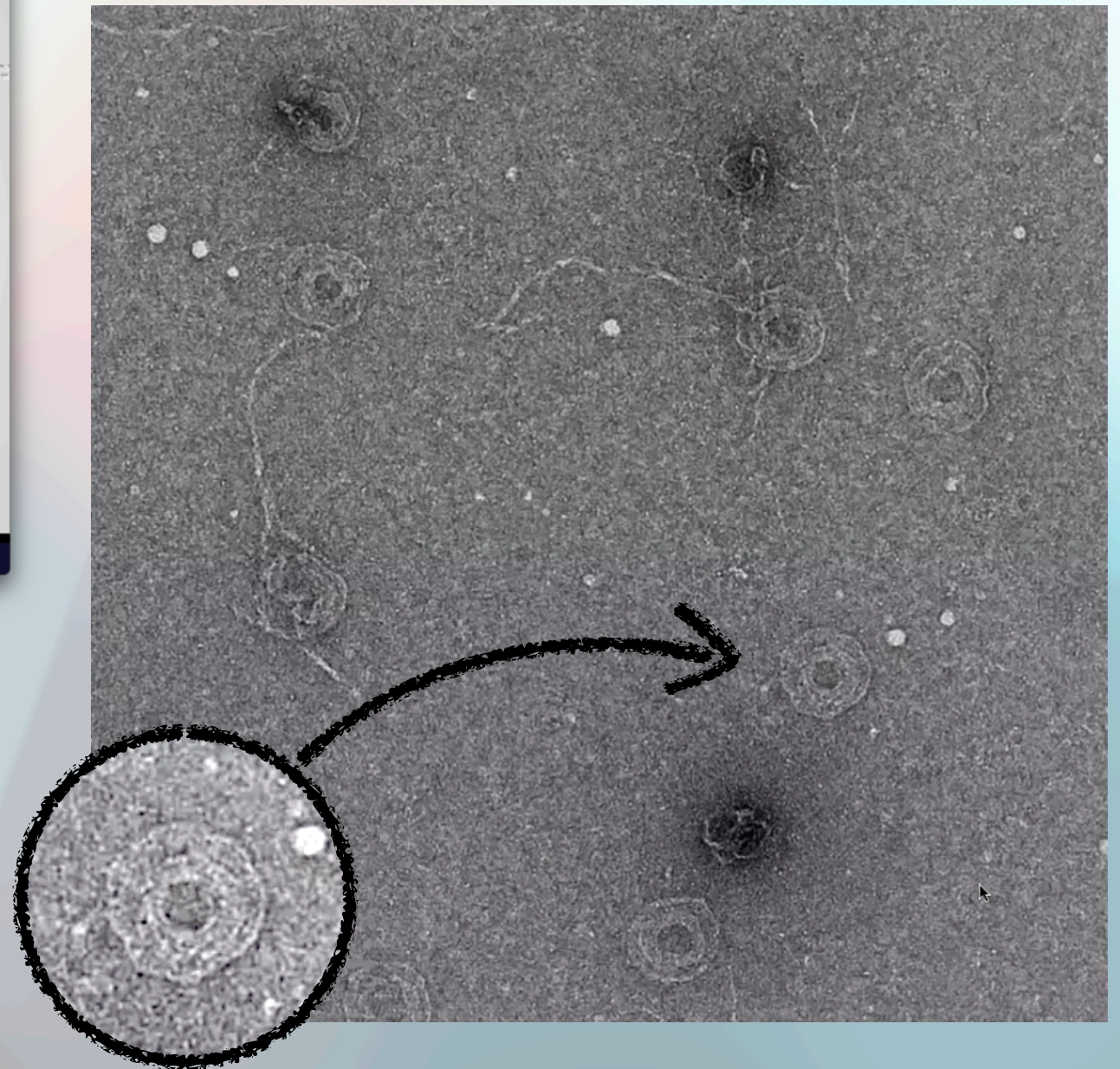
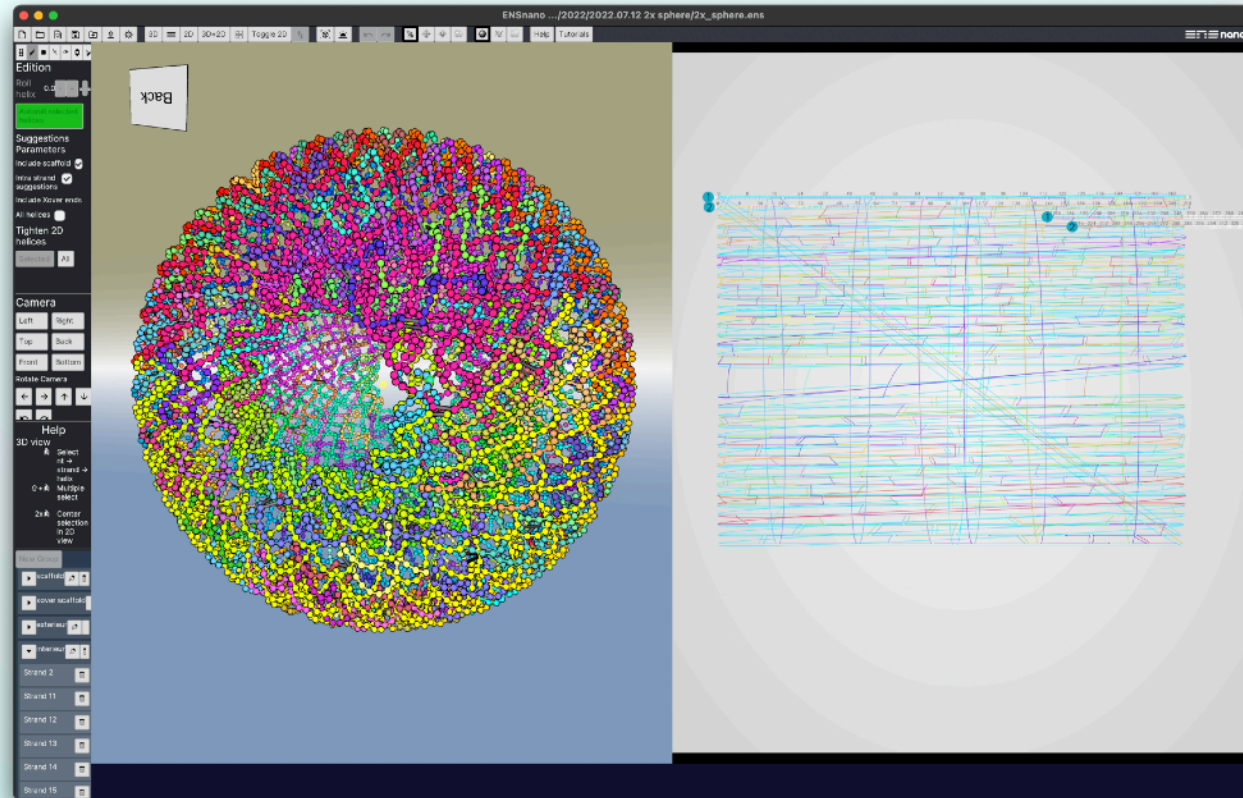
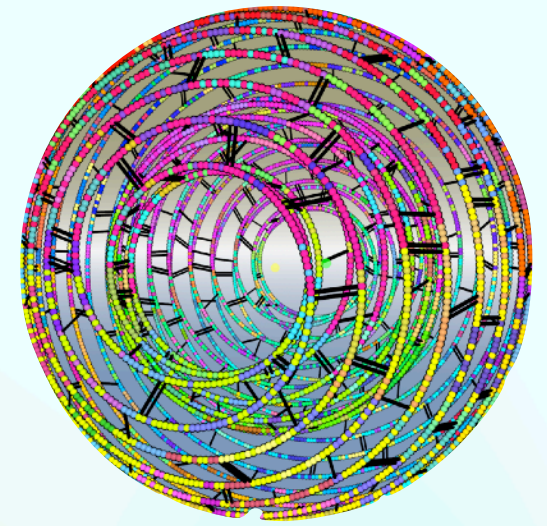
Twisted revolution surfaces



CryoEM (Gaëtan Bellot & Julie Finkel)

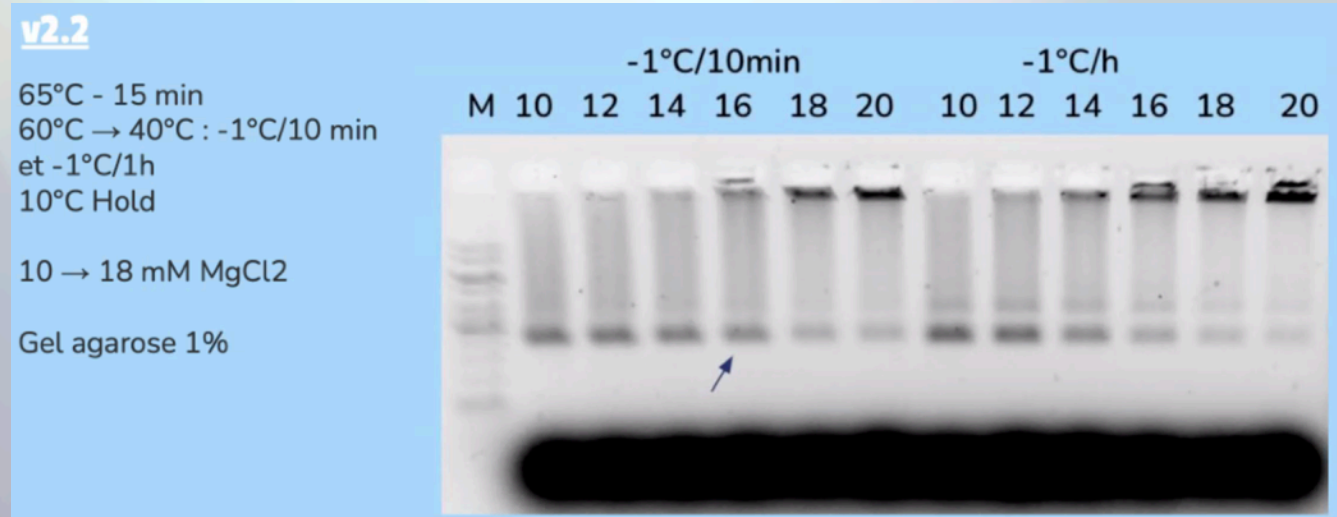
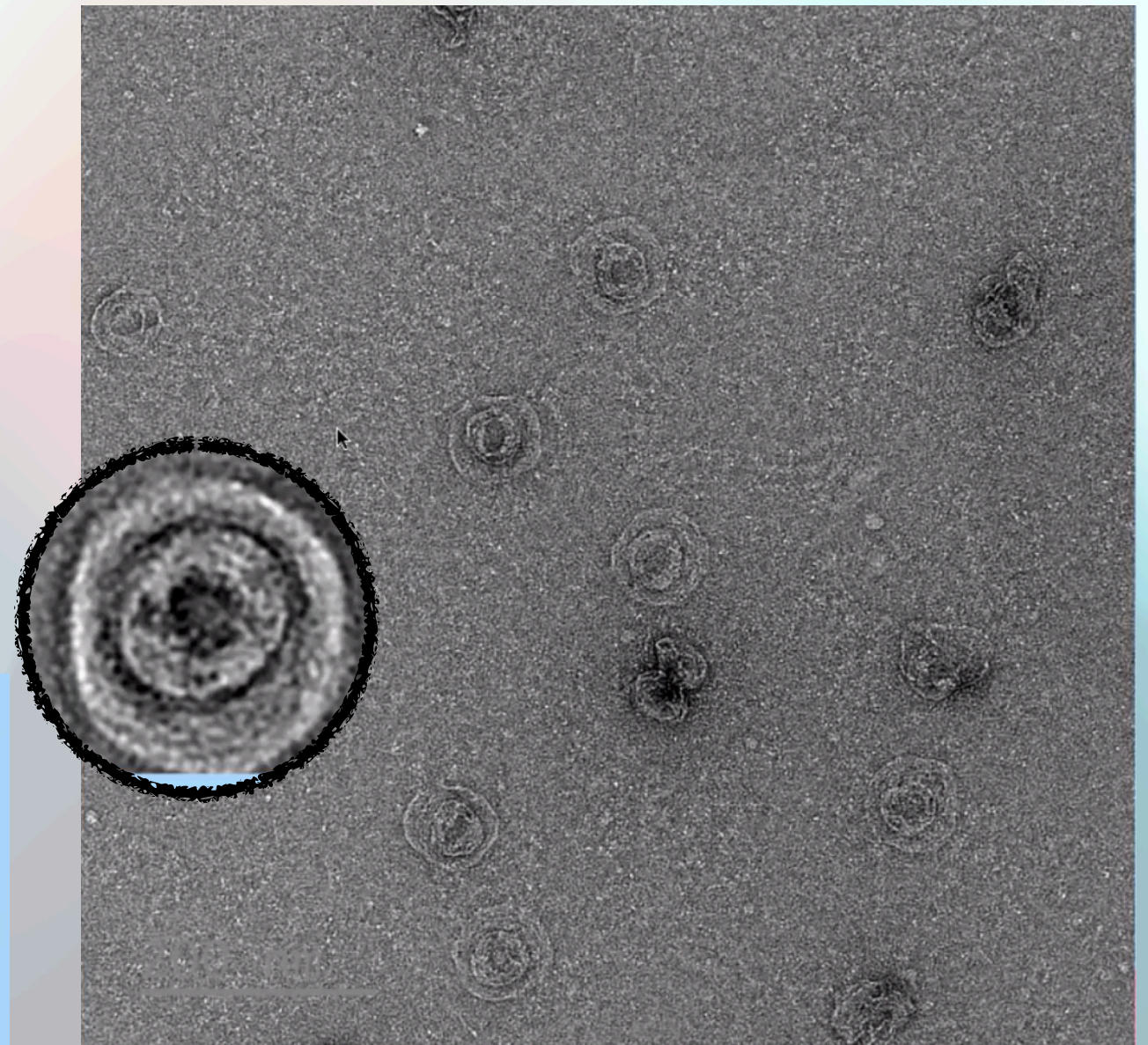
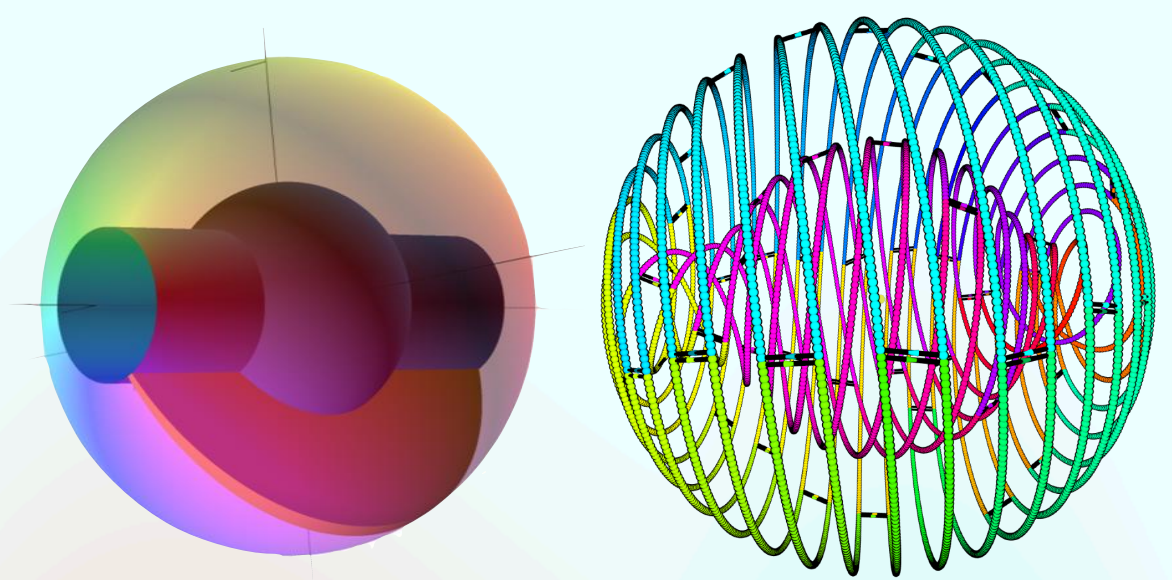
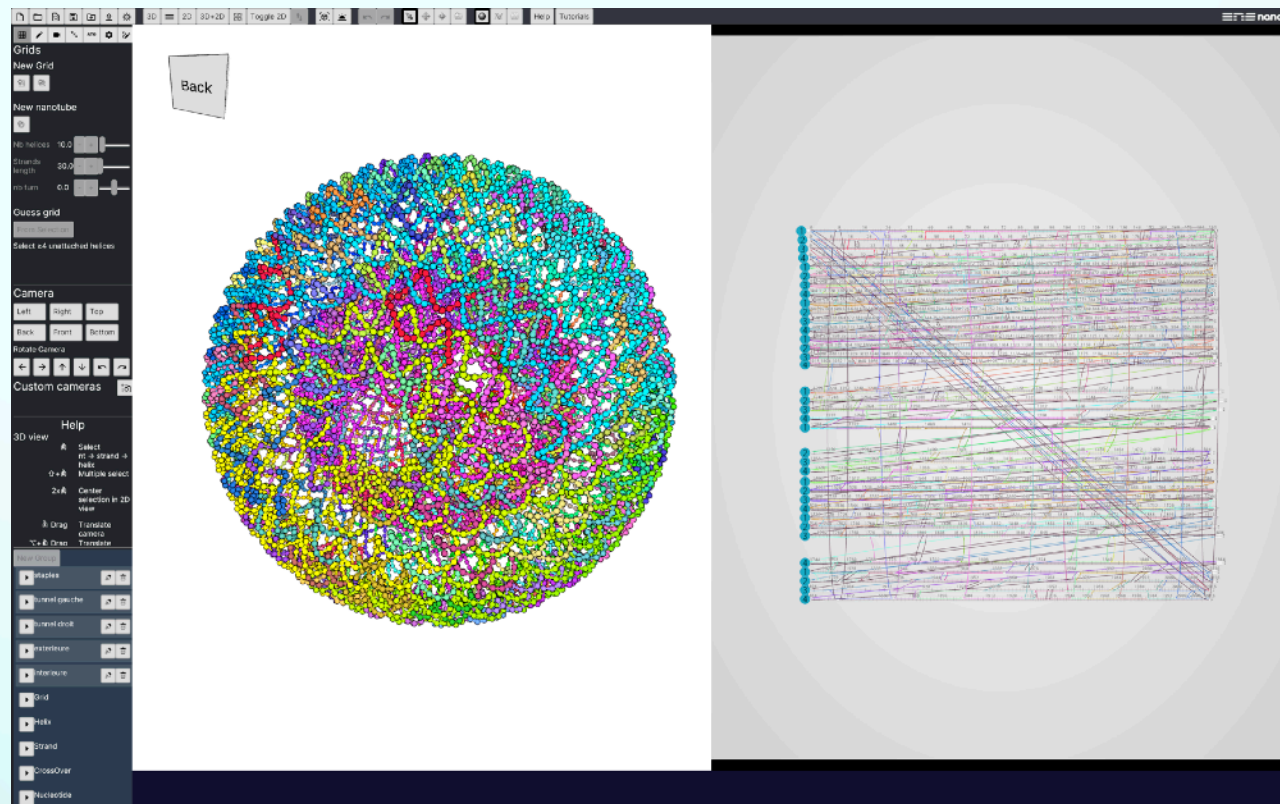
Death star I:

2 spirals



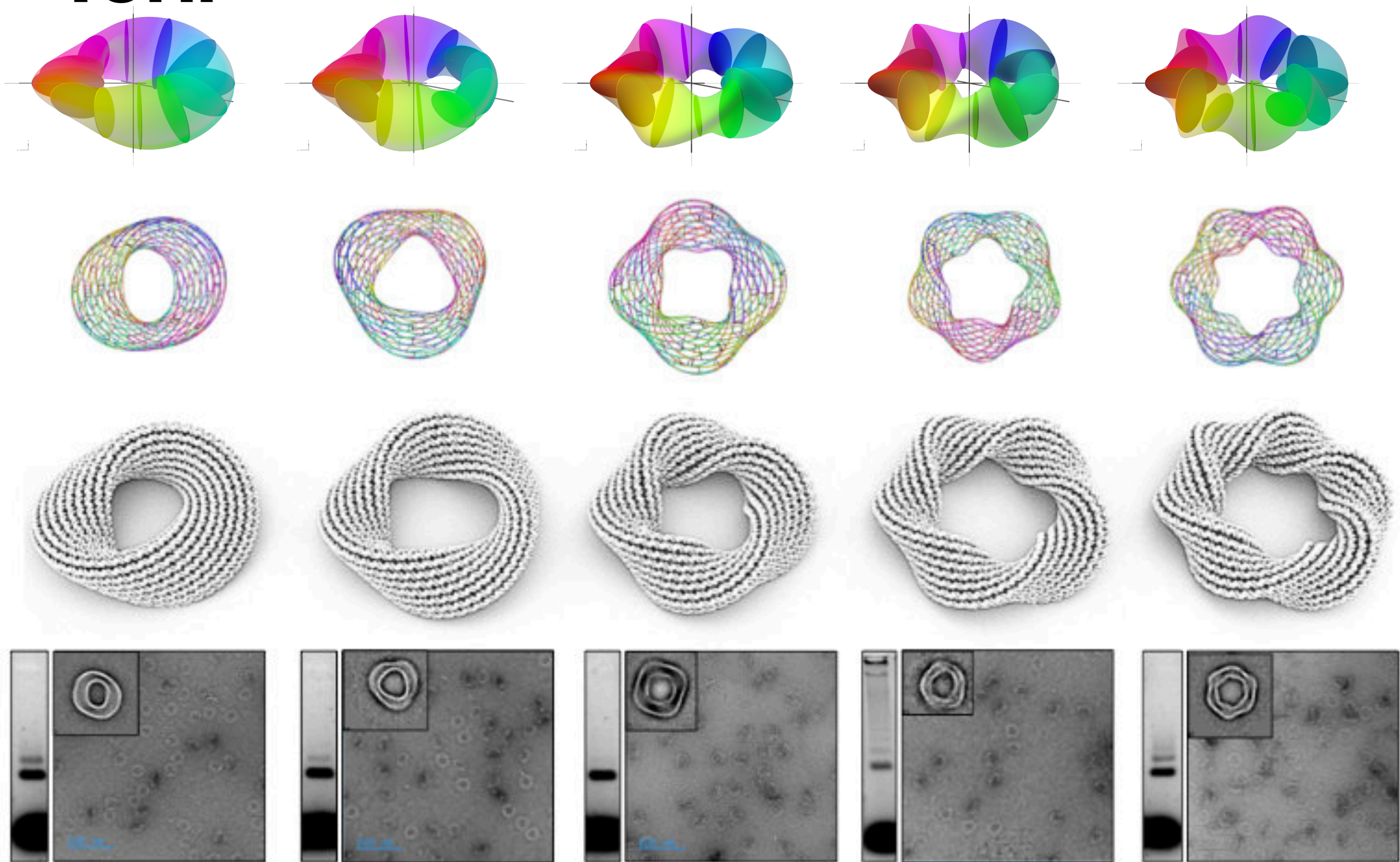
Gaëtan Bellot & Julie Finkel

Death star II: 4 spirals

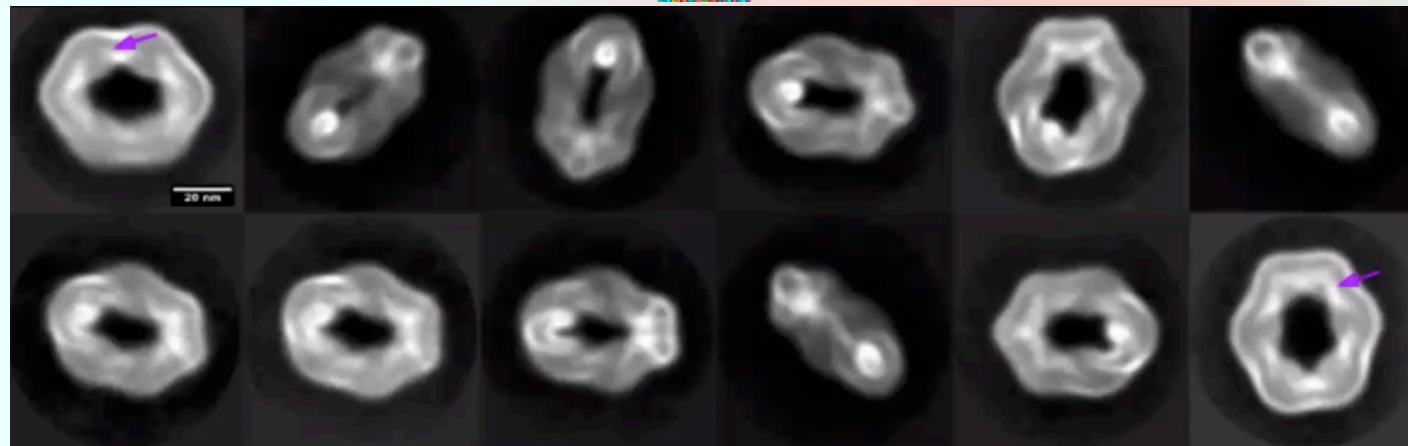
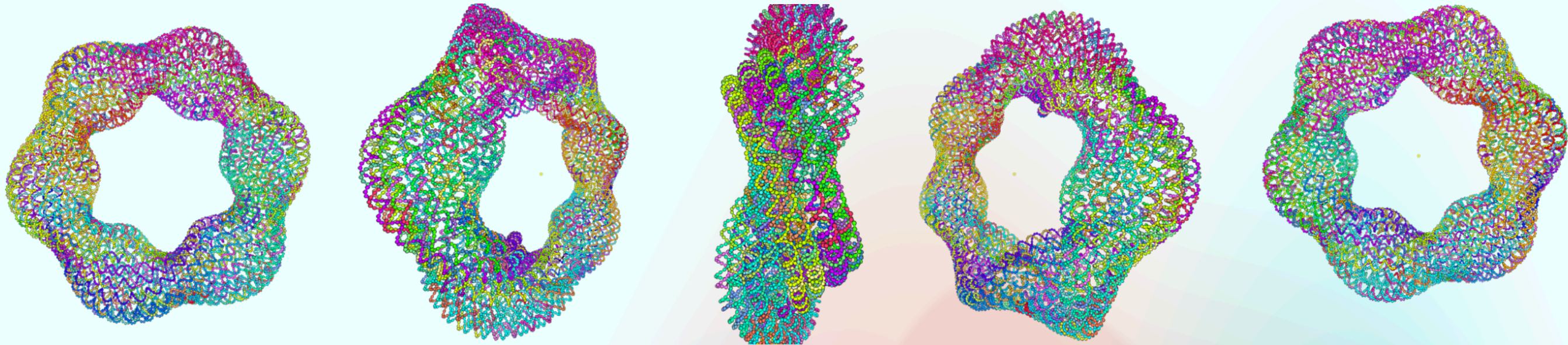


TEM by **Gaëtan Bellot & Julie Finkel**

Torii



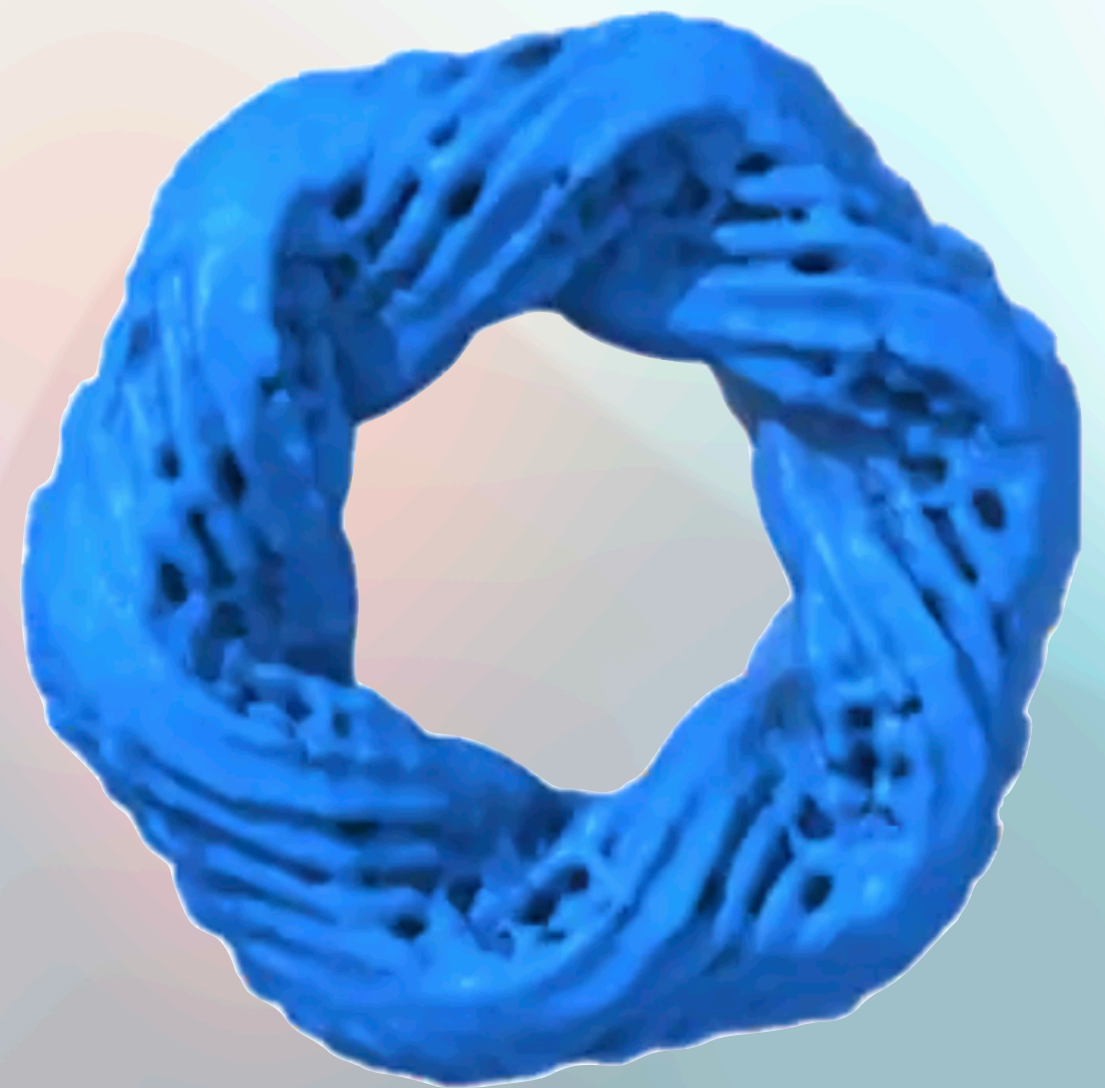
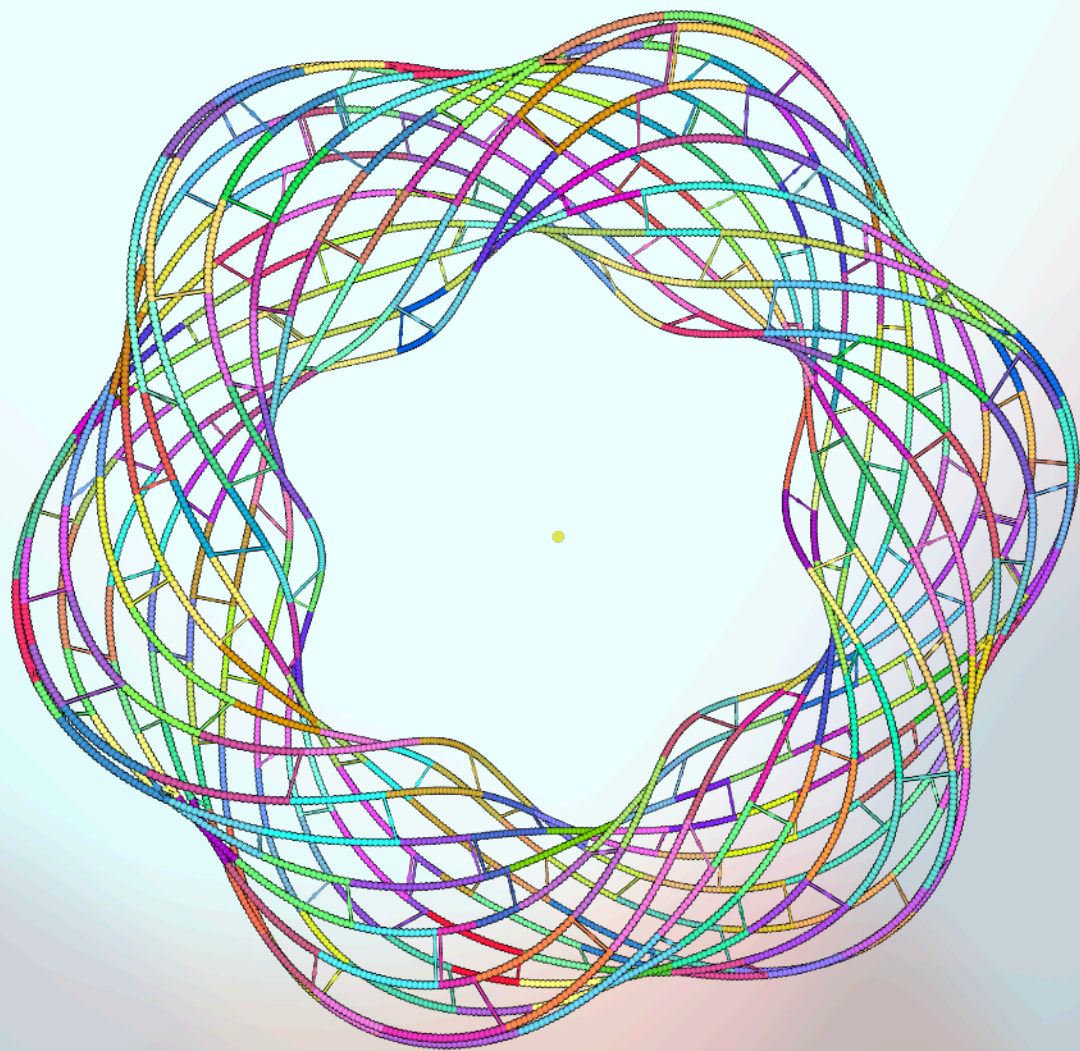
Twisted revolution surfaces



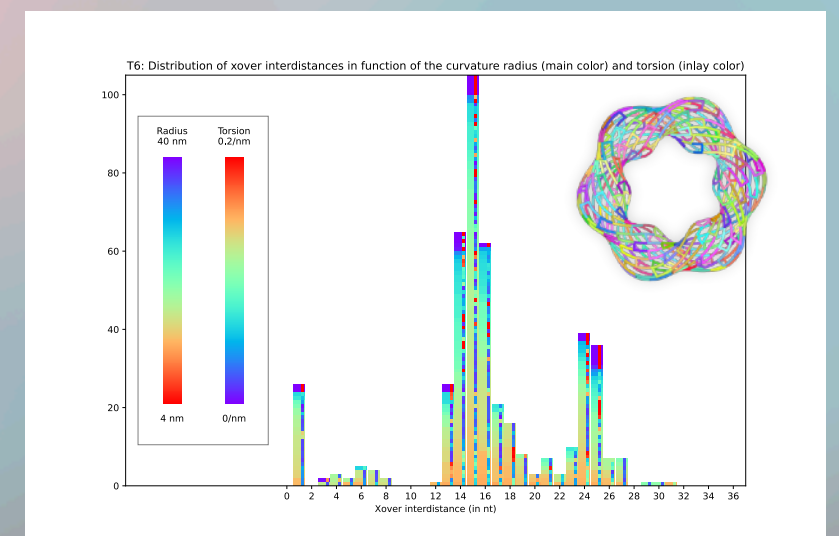
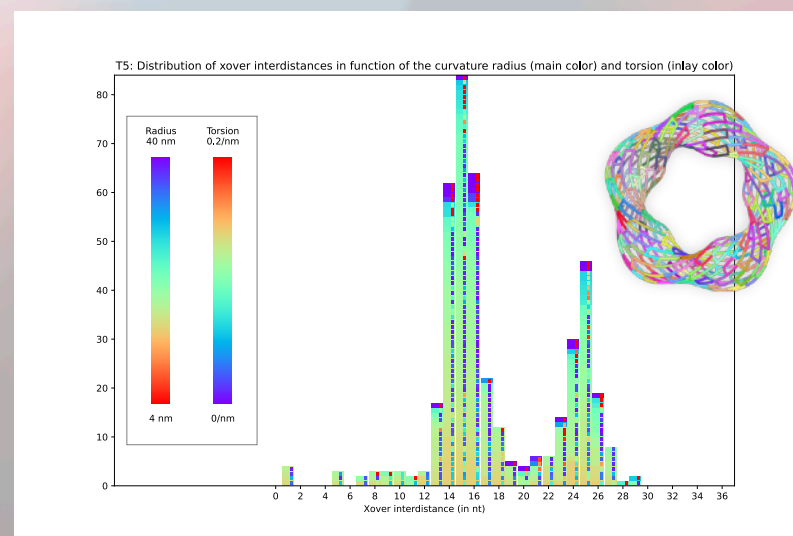
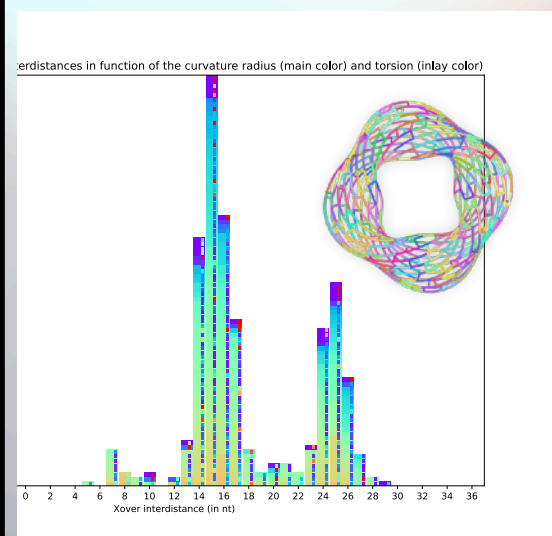
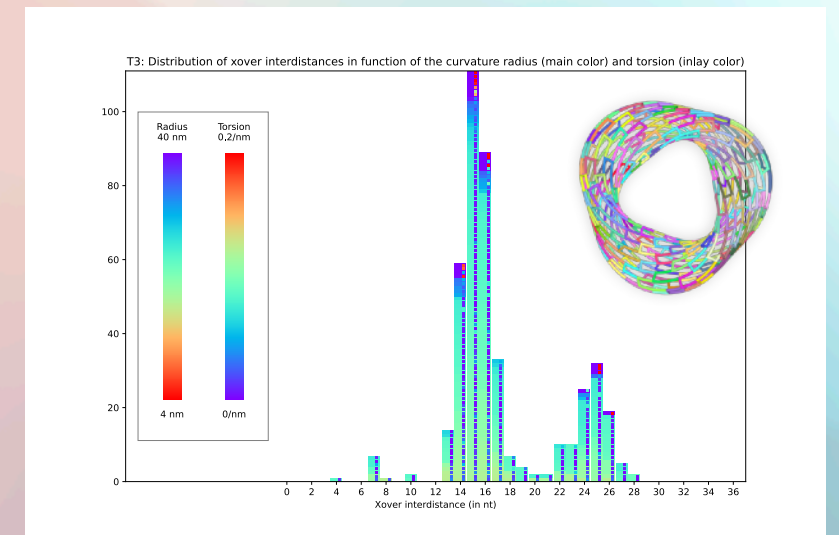
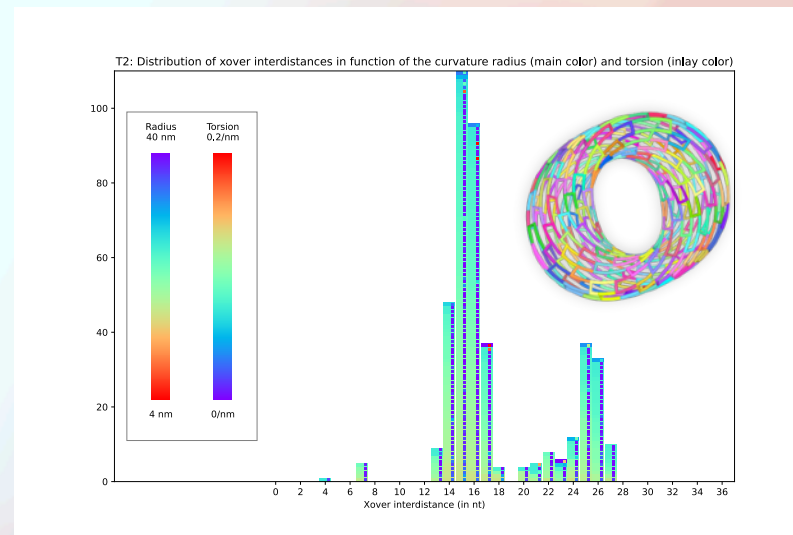
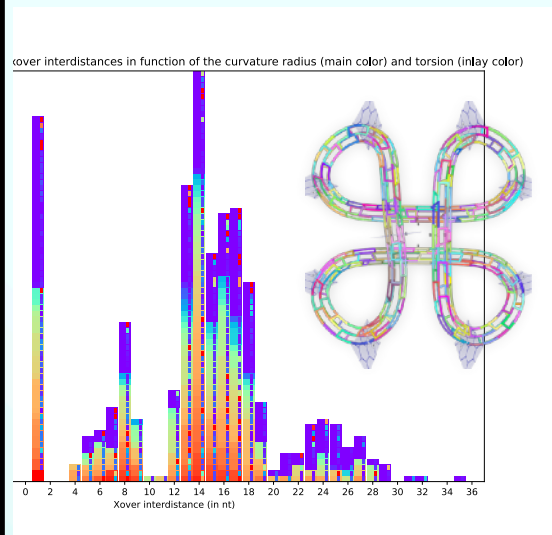
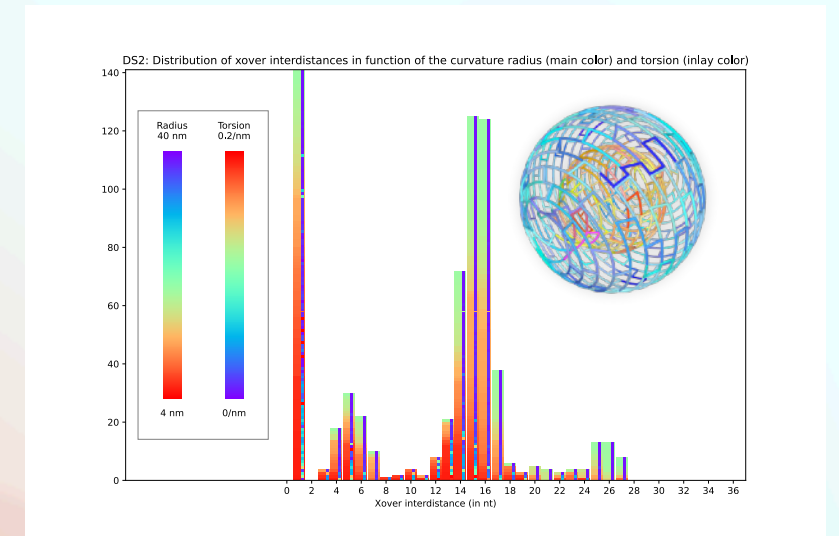
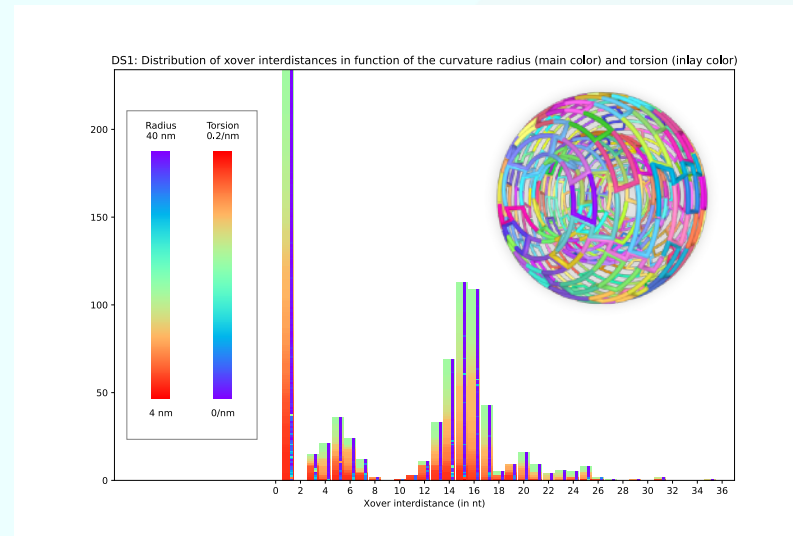
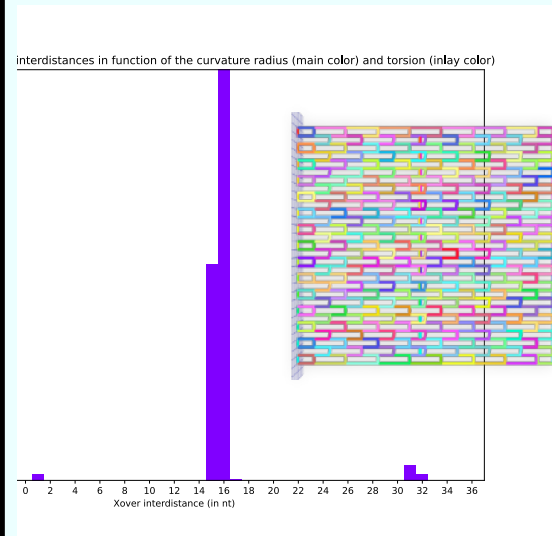
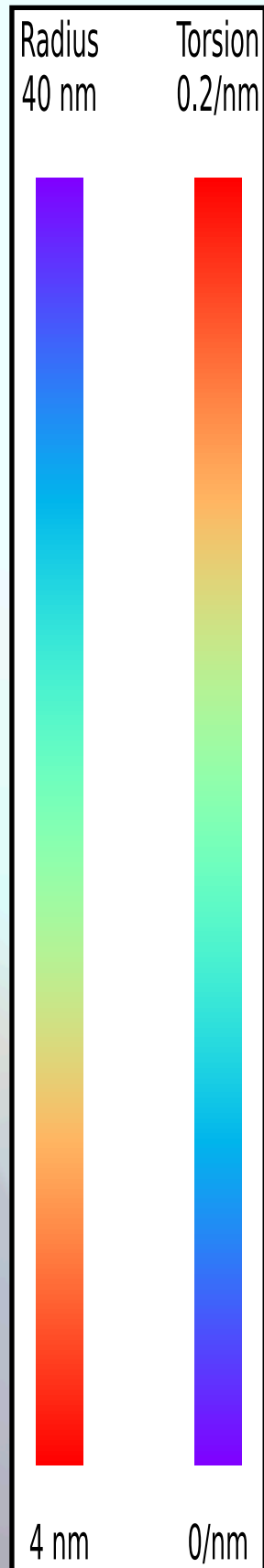
CryoEM (Gaëtan Bellot & Julie Finkel)



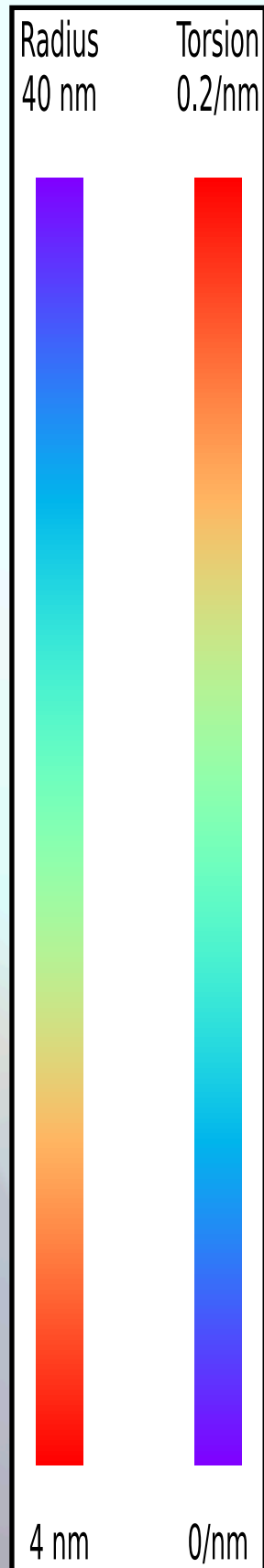
Twisted revolution surfaces



Could fixed pattern crossover spacing work?



Could fixed pattern crossover spacing work?

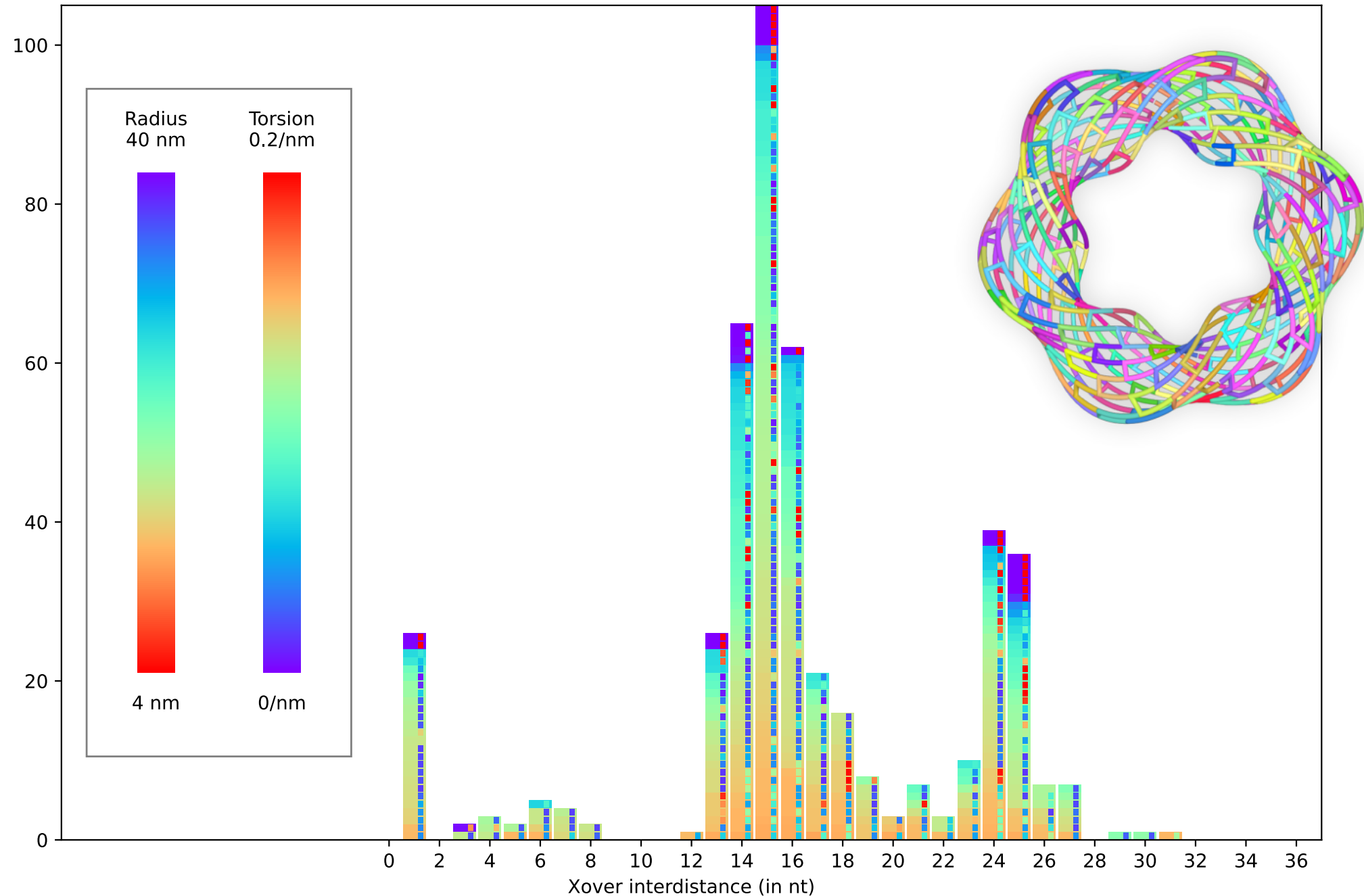


interdistances in function of the curvature radius (main color) and torsion (inlay color)

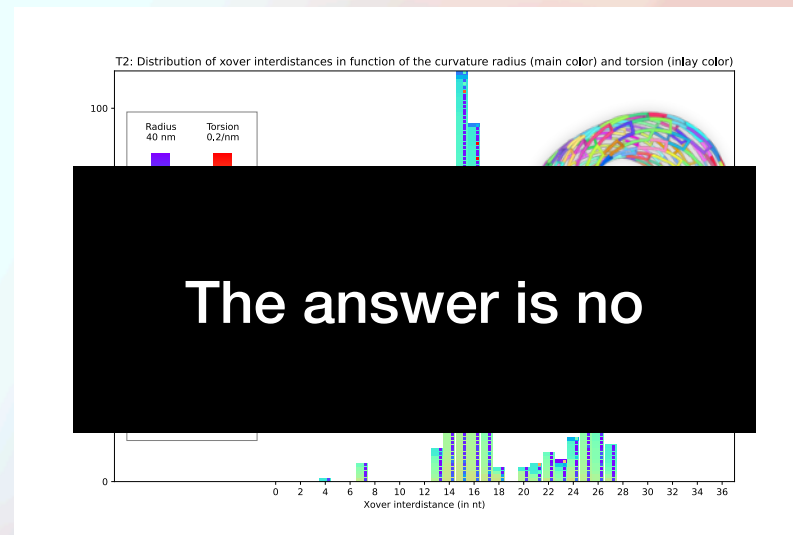
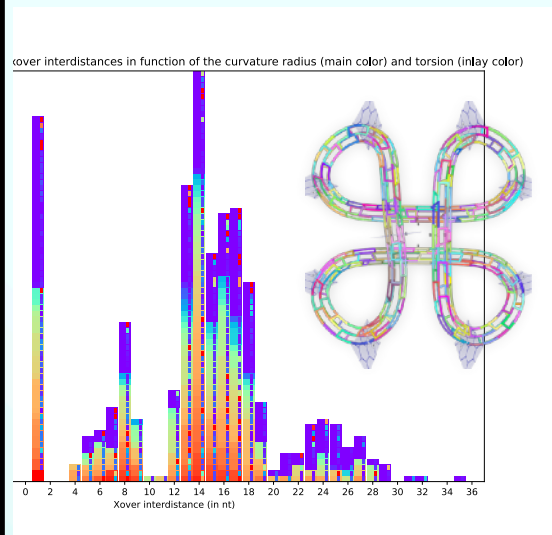
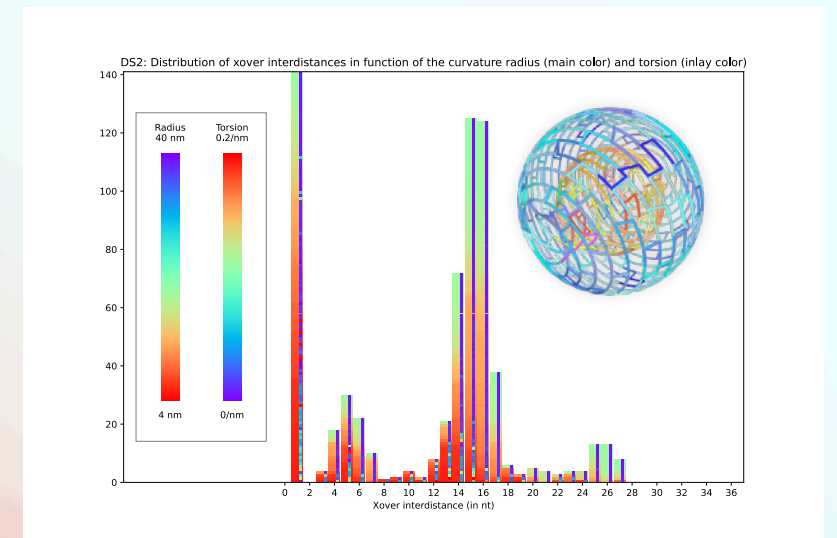
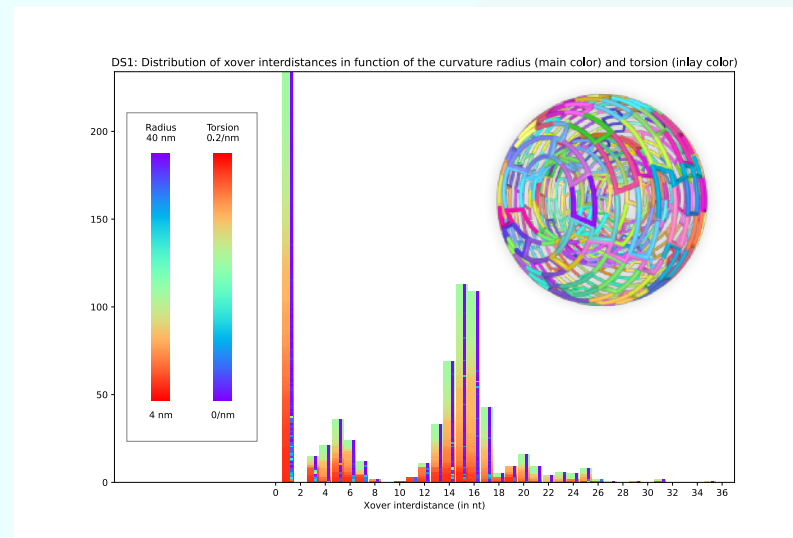
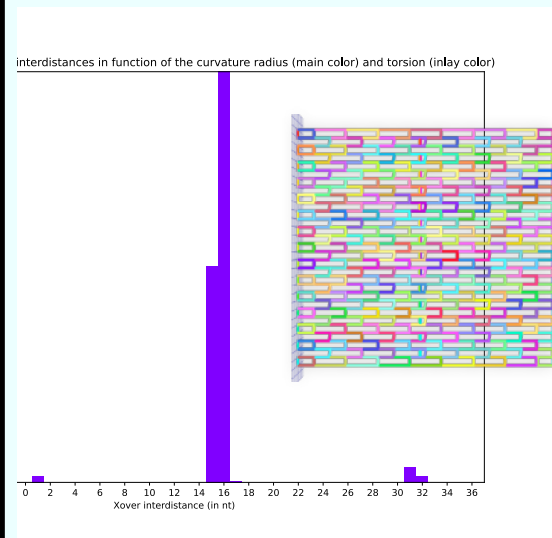
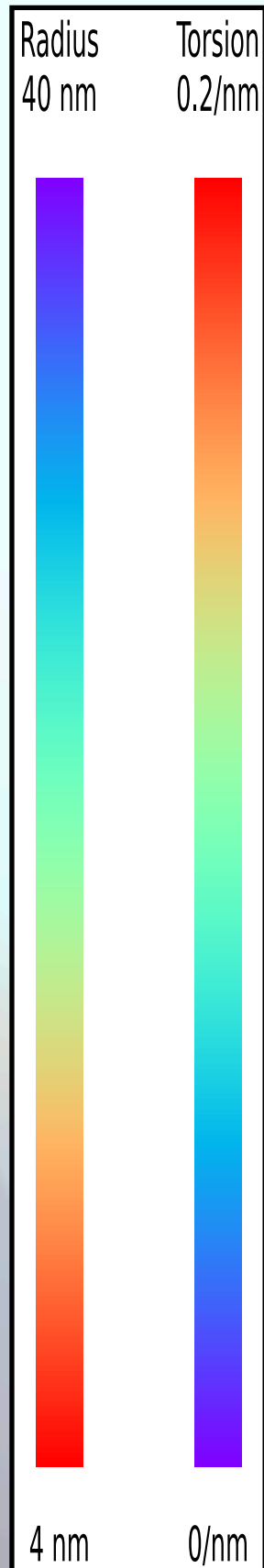
DS1: Distribution of xover interdistances in function of the curvature radius (main color) and torsion (inlay color)

DS2: Distribution of xover interdistances in function of the curvature radius (main color) and torsion (inlay color)

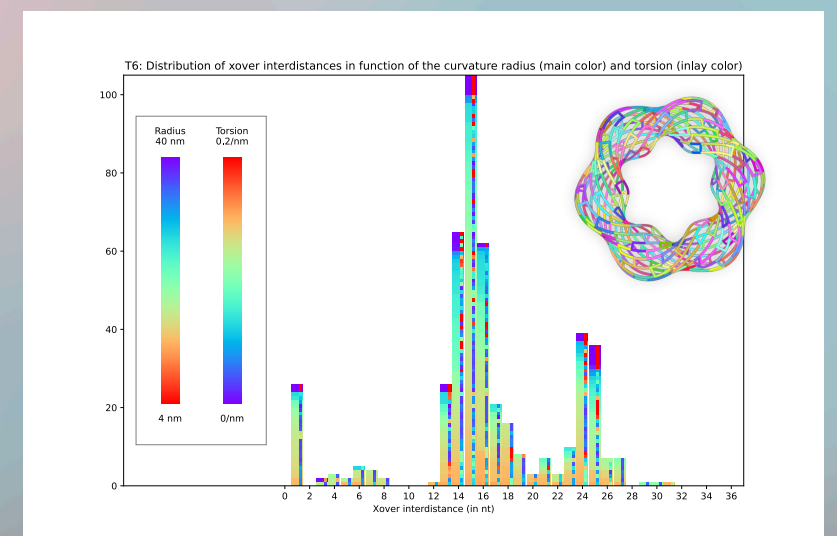
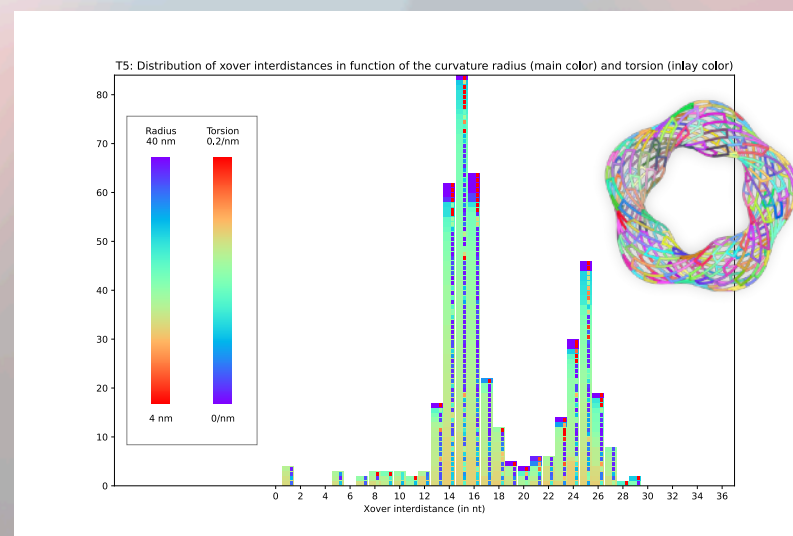
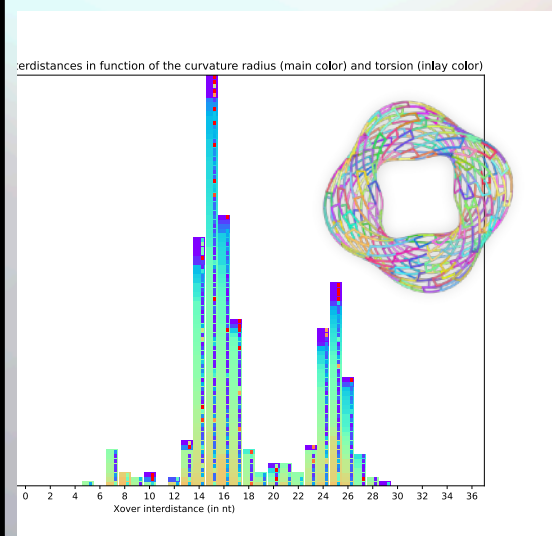
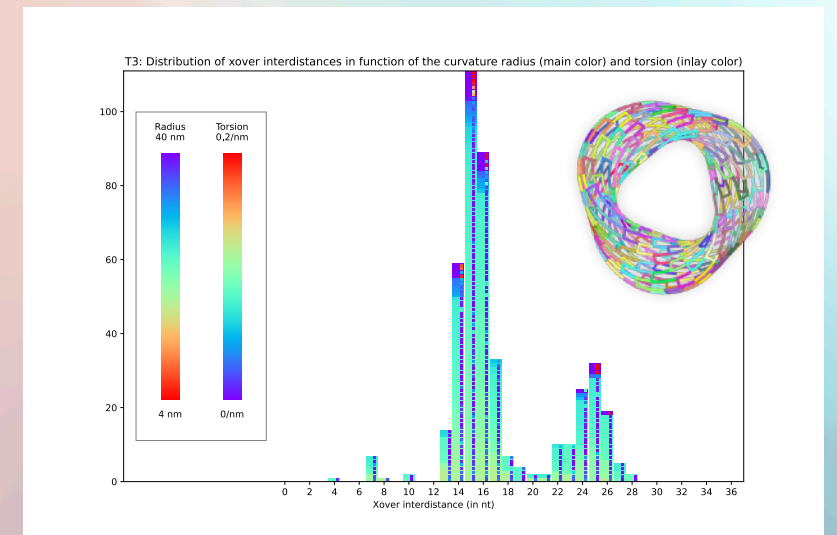
T6: Distribution of xover interdistances in function of the curvature radius (main color) and torsion (inlay color)



Could fixed pattern crossover spacing work?



The answer is no



Conclusions

Many direction to improving ENSnano

<https://www.ens-lyon.fr/ensnano>

- **User interface:** Library update and GUI reorganisation
- **Scaffolding:** Algorithmic formulation?
- **Stapling:** Local search algorithm on the way
- **Multi-component origami:** Cloning, Groups, Experiments to validate new DNA parameter optimisation,...
- **Experiments helpers:** Plate designs, all in one file
- **Dynamical shapes:** continuous deformations
- **Better and faster physics simulation feedback**

Special thanks

DNA Team in Lyon

(and some random samurai)



Octave
Hazard



Nicolas
Levy



Pierre
Marcus



Daria
Pchelina



Joris
Picot



Olivier **Gandrillon**
Catherine **Koering**
Elodie **Vallin**



Florence **Lormières**



Cendrine **Moskalenko**
Yarong **Shi**
Vincent **Dolique**



Gaetan **Bellot**
Julie **Finkel**
Allan **Mills**
Anjelica **Kucinic**

