How to Design 3D Origami
+ other stuff

Outline

- A primer to scaffolded DNA origami
  - Design
  - Methods
- CaDNAno/Cando Tutorial

What we already covered

- Bricks are domains of double-helices composed of staple strands hybridized to scaffold strands.
- Neighboring double-stranded domains are connected via inter-helix connections (immobilized Holliday junctions) formed by antiparallel crossovers.
- Crossovers can be scaffold or staple
- The density of cross-overs affects the dimensions of the object in both single- and multi-layer origami

Some tricks we've seen

- single-stranded scaffold segments
  - entropic springs in tensegrity structures (think of DNA polymer as loose spring). http://scientificcuriosity.blogspot.com/2007/12/on-rubber-bands-entropic-springs-and.html
    - See Shih et al, Self-assembly of 3D prestressed tensegrity structures from DNA.
- preventing unwanted base-stacking interactions at interfaces (loops at interfaces)
- single-stranded scaffold/staple can serve as anchors (nano breadboard).

Square, Hex or Honeycomb?

- Depends on whether your object is:
  - Container-like (may use single-layer)
  - Space-filling (may use multi-layer)

	Yield	Time	Salt
Single-layer	100%	Few hours*	20◦C, 40 mM Na^+ and 12 mM Mg2+.
Multi-layer	Depending on structure, 5-20%	Up to a week: Ex: "80°C to 60°C over the course of 80 minutes and then cooled from 60°C to 24°C over the course of 173 hours."**	buffer and salts including 5 mM Tris, 1 mM EDTA (pH 7.9 at 20°C), 16 mM $MgCl_2$

*Folding DNA to create nanoscale shapes and patterns

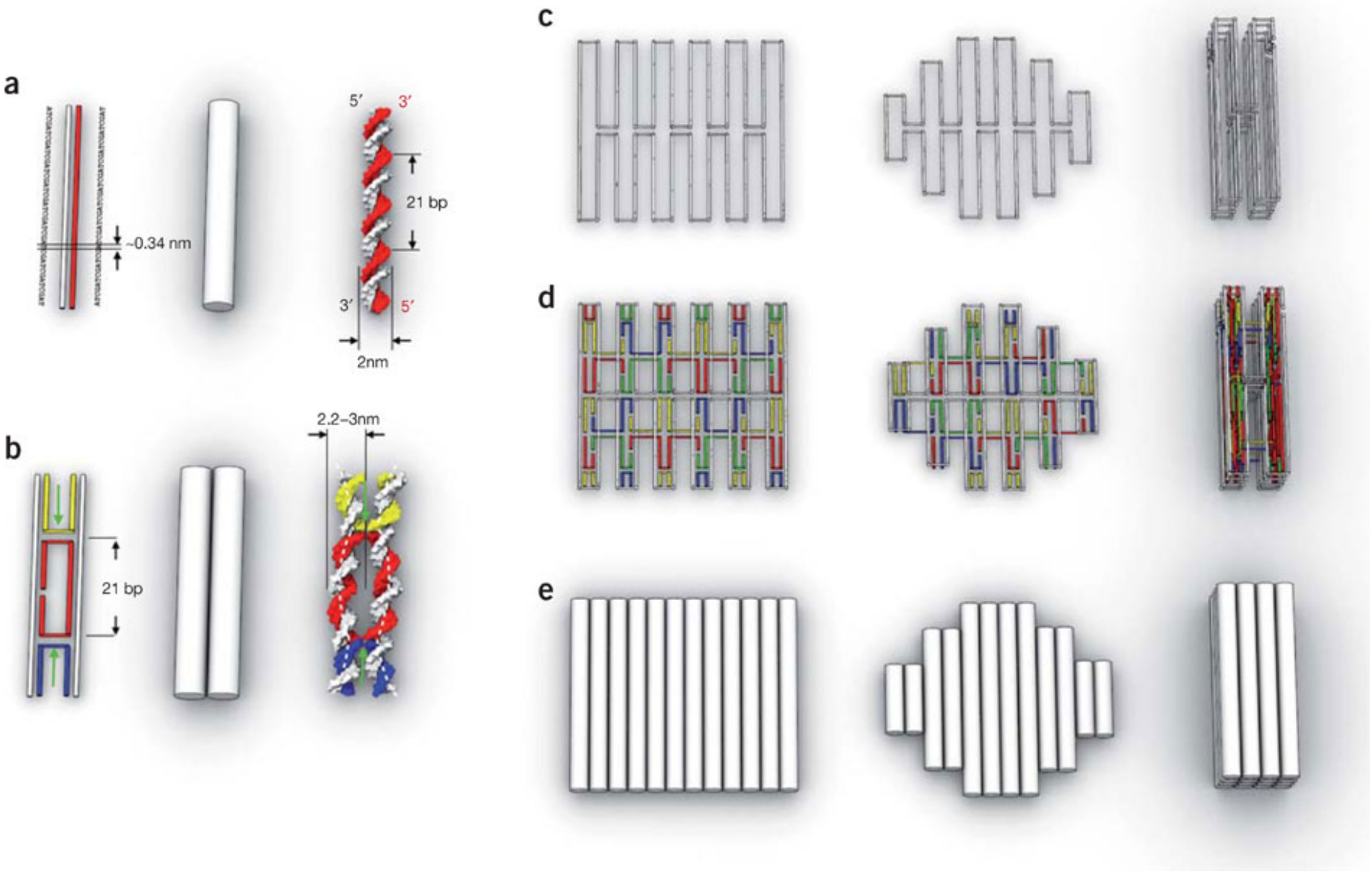
****Self-assembly of DNA into nanoscale three-dimensional shapes**

Square, Hex or Honeycomb?

- Single-layer (2D or 3D container):
  - Square (no mention of honeycomb or hex)?
- Multi-layer (3D space-filling):
  - Can use square or honeycomb (or hex, depending on your needs)
  - What's the difference?

Square, Hex or Honeycomb?

- Square:
  - four nearest neighbors per helix
  - can assume either 10.67 or 10.5 bp per turn
    - If 10.5, average spacing is 5.25 bp between crossovers (non-constant spacing intervals).
    - If 10.67, can use constant spacing of 8 bp. Between two neighbors, crossovers are spaced at 32 bps.
  - Results in twists/strains which deforms object
    - must be eliminated by non-constant deviation from 8bp.
    - can be minimized in multi-layer objects by increasing torsional stiffness in helical direction (eliminating bps to reduce helical turn length)
      - (see Shih et al, Multilayer DNA Origami Packed on a Square Lattice).
  - Densely packed objects/rectangular features
  - Requires additional effort to eliminate global twist deformations


a
~0.34 nm
5′
3′
21 bp
3′
5′
2nm
b
21 bp
2.2–3nm
c
d
e

Square, Hex or Honeycomb?

- Honeycomb lattice:
  To constrain DNA double-helix domains to this lattice configuration, you need to follow these rules:
  - assumes 10.5 bp per full turn.
  - each helix has 3 nearest neighbors
  - crossovers at 7 bp, or 240* --> 5'->3': noon, 8 pm (240*), 4 am, noon.
    - deviations cause local under/over twists + axial strain. Targeted removal/addition of bp can cause global twists/bending that can be tuned.
      - (see Shih et al, Folding DNA into twisted and curved nanoscale shapes).
  - place cross-overs between a particular pair every 21 bases, and since you have 3 neighbors, all crossovers can be spaced out at 7 bp for each neighbor.
  - creates more porous structures
  - no need to eliminate twists (with respect to crossovers)

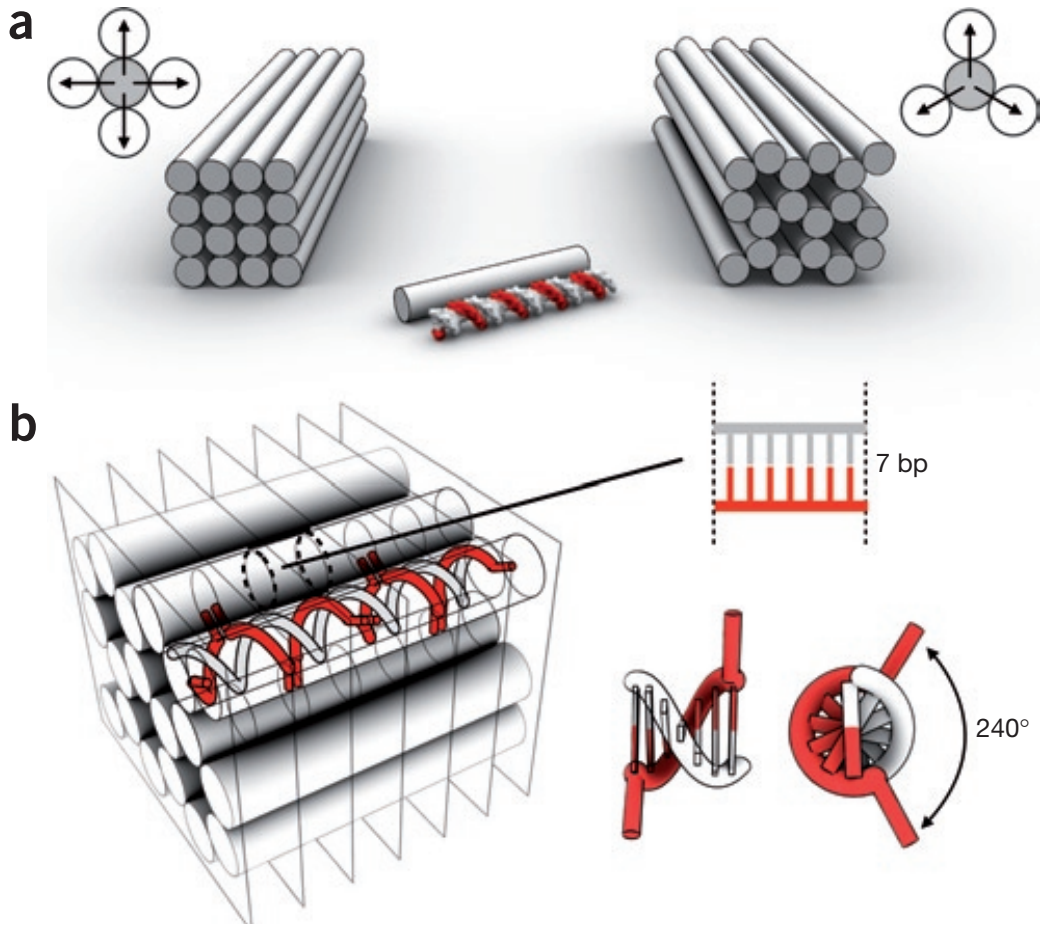


Figure 3 | Packing and cross-over spacing rules for multilayer DNA origami. (**a**) Cross-sectional view of multilayer DNA origami objects in square lattice (left) and honeycomb lattice (right) packing. (**b**) Cross-overs in multilayer objects with honeycomb lattice packing, spaced in constant intervals of 7 bp along the helical axis to link double-helical domains to each of three possible neighbors. The cross-over spacing of 7 bp complies with the natural B-form DNA twist density of 10.5 bp per turn, which corresponds to an average backbone rotation of 240° for a given strand in a DNA double-helical domain.

Square, Hex or Honeycomb?

- Hexagonal Lattice:
  - Six nearest-neighbors
  - Most densely packed
  - Best-yielding S-version (short) has crossovers every 9-bp, or 10.8 bp/turn.
  - Least susceptible to twist/compression amongst all three architectures.
  - Not available in caDNAno currently, manually designed
    - See Shih et al, Multilayer DNA Origami Packed on Hexagonal and Hybrid Lattices

Single-layer Square lattices

- Constant spacing of 16 bp between crossovers
- Likely twisted shape in solution
- Lays flat on surface (mica) due to adhesion interactions

Cross-overs, again

- Both staple and scaffold strands contribute crossovers, however:
  - For thicker objects (>2 layers), avoid global shape deformation by using 2 reference frames (for staple vs. scaffold crossovers)
    - Shifted 5-6 bps
    - Neglects major/minor grooves in B-DNA
  - For thinner objects, might need to keep track of major/minor grooves to avoid rolling up in the direction perpendicular to the dsDNA axis.
    - See Rothemund et al, Design and Characterization of Programmable DNA Nanotubes
- Alternative: *use high densities of staple cross-overs and avoid scaffold cross-overs as much as possible.*

Dimension Estimates

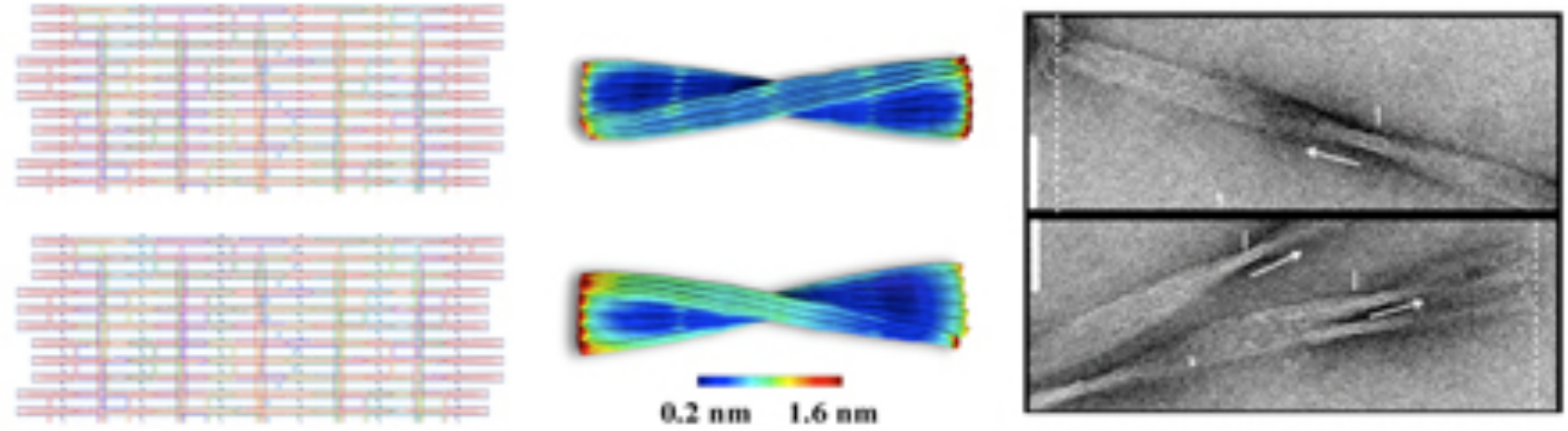
- Rules of thumb:
  - 0.34 per bp → length = (# of bps) x 0.34 nm
  - Width:
    - 2H + (H – 1)g
      - H = # of double-helical domains along axis (2 nm wide)
      - g = interhelical gap size in nm between cross-overs on the same axis.
    - Effective width of a double-helix domain: 2.1 - 2.4 nm

CanDo model

- Finite element method to compute 3D DNA origami shapes.
- Models bps as 2-node beam finite elements, representing elastic rod with geometric and material parameters.
  - Defaults:
    - Length: 0.34 nm
    - Diameter: 2.25 nm
    - Stretch modulus: 1,100 pN
    - Bend modulus of 230 pN nm^2
    - Twist modulus of 460 pN nm

CanDo result

- Deformed shape of relaxed structure
- Heatmaps of local magnitude of thermally induced fluctuations (flexibility)



CanDo limitations

- Sequence details neglected
- Does not model interhelical electrostatic repulsion
- Neglects major/minor groove details.
- Does not model tensegrity-like structures

Origami object stability

- Do origami objects remain folded?
  - Heat
  - Solution conditions
  - Nucleases

Design steps

1. Conceive target shape
2. Design layout, evaluate design and determine staple sequences
3. Prepare scaffold DNA/synthesize stapes
4. Pool subsets of concentration-normalized oligonucleotides
5. Run molecular self-assembly reactions
6. Analyze folding quality/purify objects
7. Single-particle based structural analysis

1. Conceive target shape

Single or multi-layer?

Square or honeycomb (or hex or hybrid?)

Can divide into modules and design.

2. Design layout, evaluate design and determine staple sequences

"In practice, multiple scaffold-staple layouts may have to be made for the same target object to identify a solution that yields well-folded objects." → Trial and error

- Might require site-directed attachments or fluorescent dyes.

3. Prepare scaffold DNA/synthesize stapes

- The quality of folding of DNA origami objects may depend on:
  - the scaffold sequence and
  - the particular cyclic permutation used in the design.
- Preparing single stranded DNA through:
  - Supplementary Protocol I: growing phage + purification
  - Enzymatic digestion of a strand
  - Can use dsDNA
- "DNA origami objects are assembled with, on the average, 40-nucleotide-long staple molecules; individual staples may range in length from 18 nucleotides to 50 nucleotides"

4. Pool subsets of concentration-normalized oligonucleotides

“Equal amounts of concentration-normalized staple oligonucleotides belonging to a structural module are mixed to form a common pool. “

5. Run molecular self-assembly reactions

"The goal of the assembly reaction is to reach a minimum energy state at conditions where the minimum corresponds to the target structure."

- Single-layer objects self-assemble faster than multilayer objects.
- The assembly of multilayer objects can proceed along a multitude of pathways that may not necessarily lead to the fully folded target structure but to partially folded dead ends (kinetic traps) in which parts of the structure need to dissolve before assembly can proceed.
- Single-layer objects can be assembled by briefly heating the mixture of scaffold and staples to 80 °C, followed by annealing at room temperature during a few hours.
- Multilayer structures have been observed to require annealing over several days.
- Isothermal chemical denaturation and renaturation is an alternative to thermal annealing (formamide).
- Folding DNA origami objects by sequential addition of staples to scaffold or by tuning the staple length or sequence composition remain unexplored methods by which the user may direct the system along assembly pathways devoid of substantial kinetic folding traps.

5. Run molecular self-assembly reactions

A folding reaction contains:

1) scaffold DNA
2) staple DNA
3) water
4) pH-stabilizing buffer
5) additional ions.

- Scaffold and staple DNA are typically added such that each staple is present in a defined stoichiometry relative to the scaffold in five-to tenfold excess → Exact stoichiometries seem not to matter.
- Scaffold strands need not be purified
- Different staple-scaffold stoichiometries may need to be tested.
- Yield of assembly of multilayer objects is sensitive to $MgCl_2$ concentration.

A detailed protocol for setting up folding reactions is available in **Supplementary Protocol 2.**

6. Analyze folding quality/purify objects

- Analysis of the quality of folding of DNA origami objects and purification of a desired species can be accomplished with agarose gel electrophoresis.
- Agarose gels and the running buffer should contain magnesium.
- For multilayer objects it has been found that for a given object, the objects with lowest defect rate as judged by direct imaging by TEM were those that migrate with the highest speed through a 2% agarose gel.
- Thus, assembly reactions can be optimized by searching for conditions that yield the fastest migrating species.
- The yield for agarose gel purification varies with object shape.
- A protocol for gel electrophoresis and purification is available in **Supplementary Protocol 3.**

7. Single-particle based structural analysis

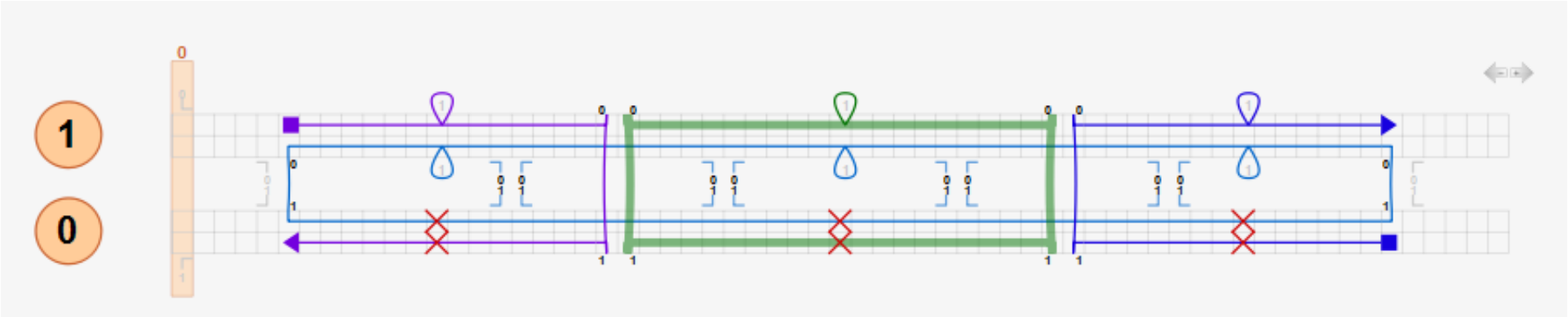
- DNA origami objects may be imaged with negative-stain or cryogenic TEM and with atomic force microscopy (AFM).
- Shape heterogeneity can be assessed on a particle-by-particle basis. Image processing can help to identify systematic structural flaws or to reconstruct 3D models from single-particle TEM data.
- Negative-stain TEM with 2% uranyl formate as staining agent is a convenient tool for imaging multilayer objects.
- Protocols for setting up negative-stain TEM and AFM experiments (with the protocol for the latter contributed by P. Rothemund, Caltech) are available in **Supplementary Protocols 4** and **5.**

What to take into consideration when designing

- Shape
- Scaffold
- Staples
- Crossover spacing
- Sequence design

1. Preparing an input design file using the caDNAno

In this tutorial, we will use the .json file for a 53 basepair long two-helix bundle design where three insertions and deletions exist in each helix. The corresponding .json file can be downloaded here.



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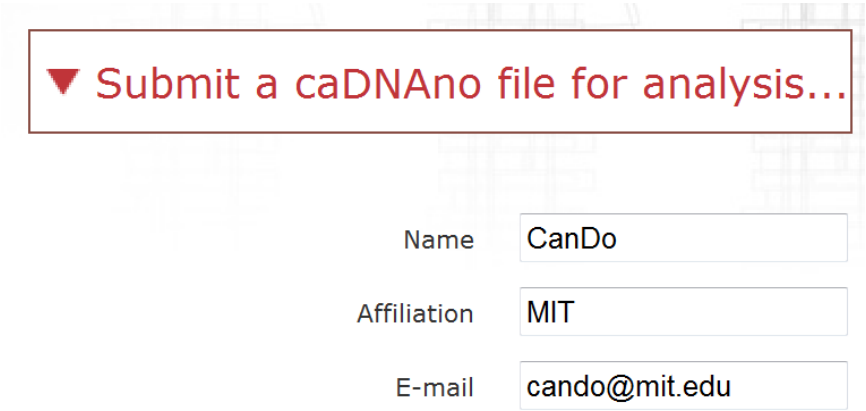
2. Filling out the submission form

1. Click the red box (Submit a caDNAno file for analysis...) to expand the submission form

2. Enter user information (Name, Affiliation, and E-mail address).



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2. Filling out the submission form

3.DNA geometry

Default values for average B-form DNA geometry are pre-entered. Alternatively, users may enter their own values.

DNA geometry
(Use pre-entered default values or enter your own)

Axial rise per base-pair [nm]	0.34
Helix diameter [nm]	2.25

4.DNA mechanical properties

Default values for average B-form DNA mechanical properties are pre-entered. Users may enter their own values. Nicks are modeled by reducing backbone bending and torsional stiffness by a factor of 100 by default (corresponding to the default nick stiffness factor, 0.01) whereas stretching stiffness is retained at double-helix values. It is not recommended to use a nick stiffness factor less than the default value as it may result in much slower or no convergence of the analysis.

DNA mechanical properties
(Use pre-entered default values or enter your own)

Axial stiffness [pN]	1100
Bending stiffness [pN nm^2]	230
Torsional stiffness [pN nm^2]	460
Nick stiffness factor	0.01

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2. Filling out the submission form

5. Model resolution

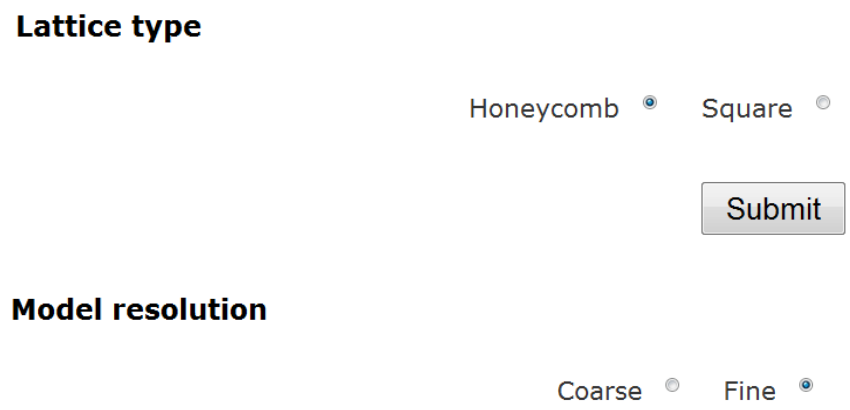
CanDo analysis is performed at the coarse model resolution by default. Users have an option to use the fine model resolution that computes the shape and flexibility at a single basepair resolution. However, the use of the coarse model is appropriate to obtain quick feedback for initial designs as it significantly reduces the computation time. Here we choose the fine resolution for purposes of this tutorial.

6. caDNAno (.json) file

Browse to the location of your caDNAno design file.

7. Lattice type

Users must choose the lattice type, either honeycomb or square.



3. Press Submit

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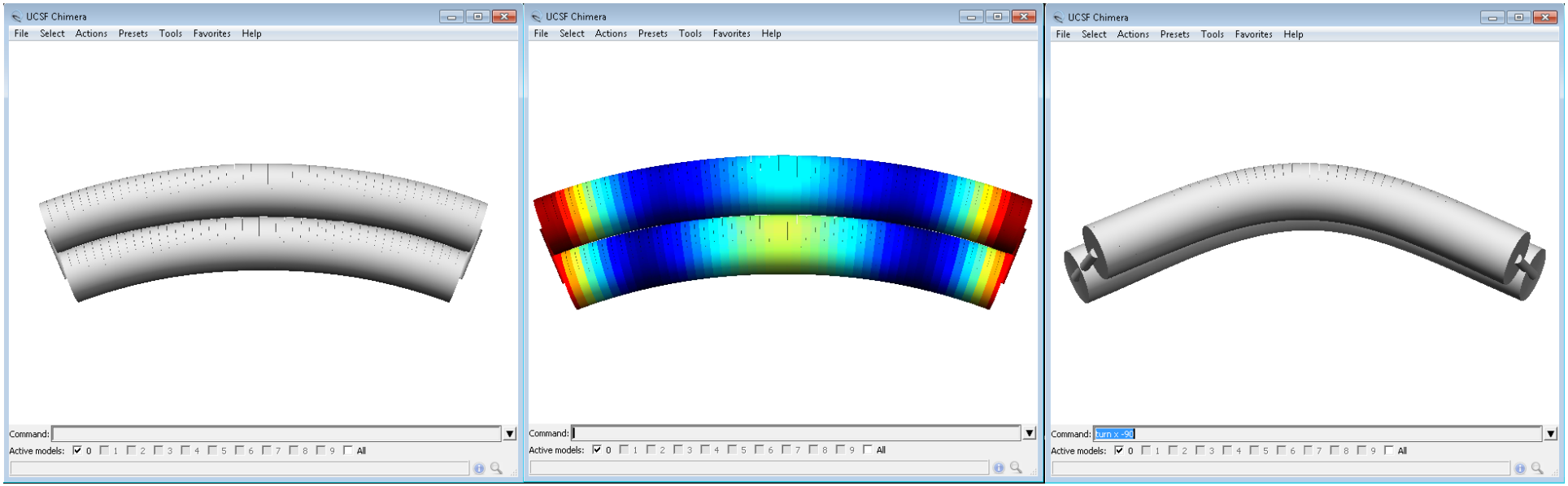
4. Viewing the results

Once the CanDo analysis is completed, users can download a single zip file containing the following results on the result page.

1. The deformed structure in unicolor (****_deformedShape.bild)
2. The deformed structure with root-mean-square thermal fluctuations indicated in color superimposed (****_RMSF.bild)
3. Movies of thermal fluctuations in three orthogonal views (e.g. fluctuations_view1.avi)
4. Movies of solution shape calculation (e.g. loadsteps_view1.avi)
5. The lowest five normal modes of the deformed structure at 1 k_BT, 2 k_BT, 3 k_BT, and 10 k_BT in unicolor (e.g. ****_Mode1_1KbT.bild)



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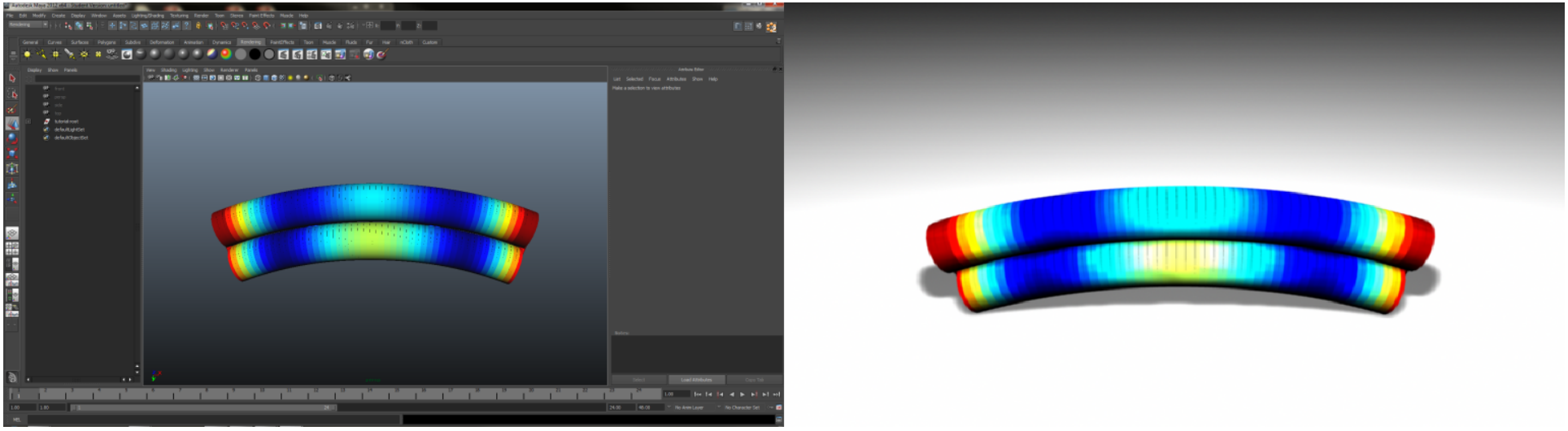
4. Viewing the results

Users may export BILD format into VRML format for use in other visualization programs including the Autodesk Maya for high-quality rendering. For example, a file conversion procedure for use with Autodesk's visualization program Maya is as follows.

1. Open the BILD file in UCSF Chimera (File > Open…).
2. Export the file as a VRML file (File > Export Scene…, select file type to VRML [.wrl, vrml]).
3. Convert the VRML file into a Maya Ascii (.ma) file by executing "wrl2ma.exe -i {input file name, ****.wrl} -o {output file name, ****.ma}" in command-line. The executable file, wrl2ma.exe, is located in bin directory (e.g. C:\Program Files\Autodesk\Maya2012\bin).
4. Open the Maya Ascii file using the Autodesk Maya. The figure below shows the deformed structure imported into Maya (left) and an example rendered image (right).



Recommended Reading

- Submicrometre Geometrically Encoded Fluorescent Barcodes Self-assembled from DNA
- Controlling the Formation of DNA Origami Structures with External Signals
- A Logic-Gated Nanorobot for Targeted Transport of Molecular Payloads

Arbona et al – Modelling the folding of DNA origami

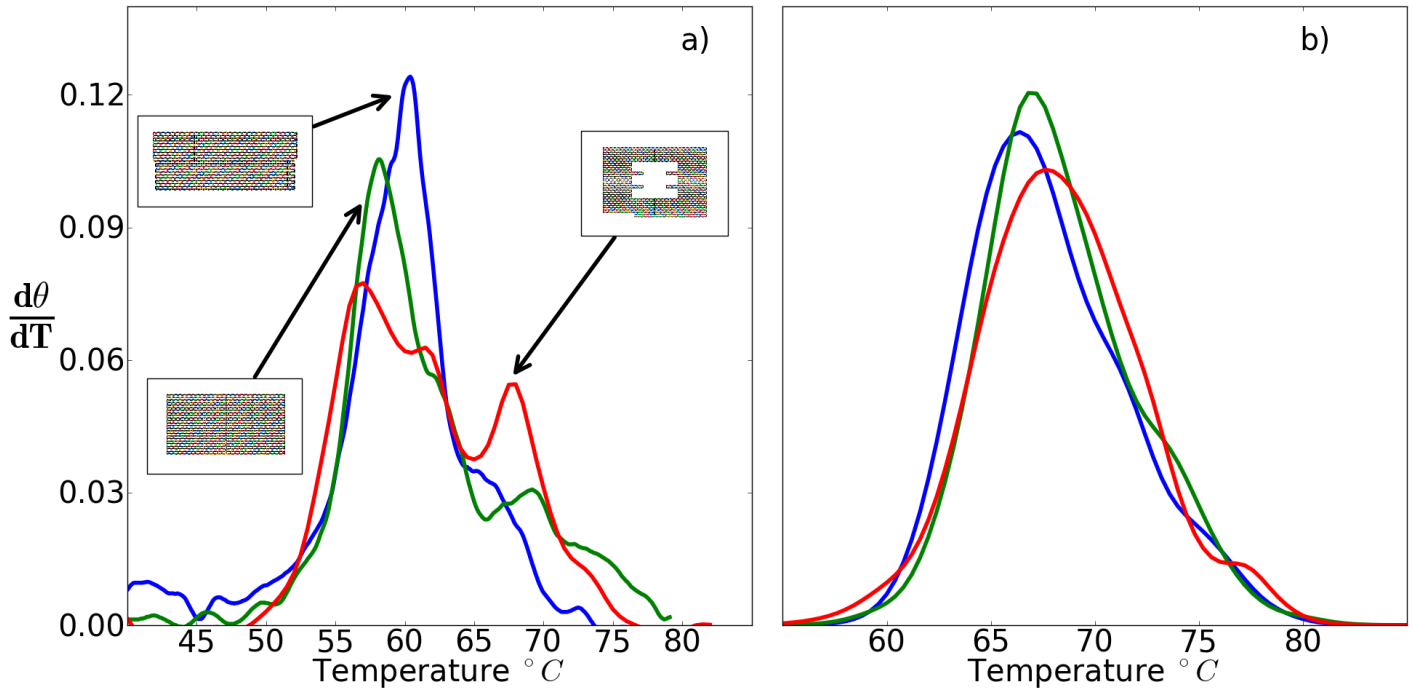


Fig. 1. (a)Derivative $d\theta/dT$ of the degree of pairing with respect to temperature for the three DNA origamis represented in the insets. (b) $d\theta/dT$ for a model where

Arbona, J. M., Elezgaray, J., & Aimé, J. P. (2011). Modelling the folding of DNA origami. arXiv preprint arXiv:1111.7130. Retrieved from http://arxiv.org/abs/1111.7130

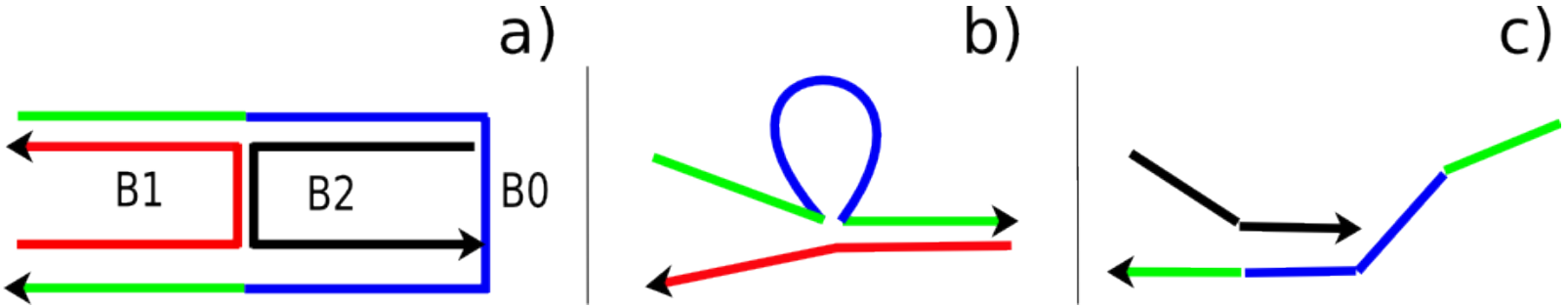


Fig. 2. (a) Schematic representation of the connectivity of the small origami.(b) B1 staple is in the 'outer' position,(c) B2 staple in the 'inner' position. (b) and (c) show that the binding of staples in the outer (b) or inner (c) positions are very different.

Arbona, J. M., Elezgaray, J., & Aimé, J. P. (2011). Modelling the folding of DNA origami. arXiv preprint arXiv:1111.7130. Retrieved from http://arxiv.org/abs/1111.7130

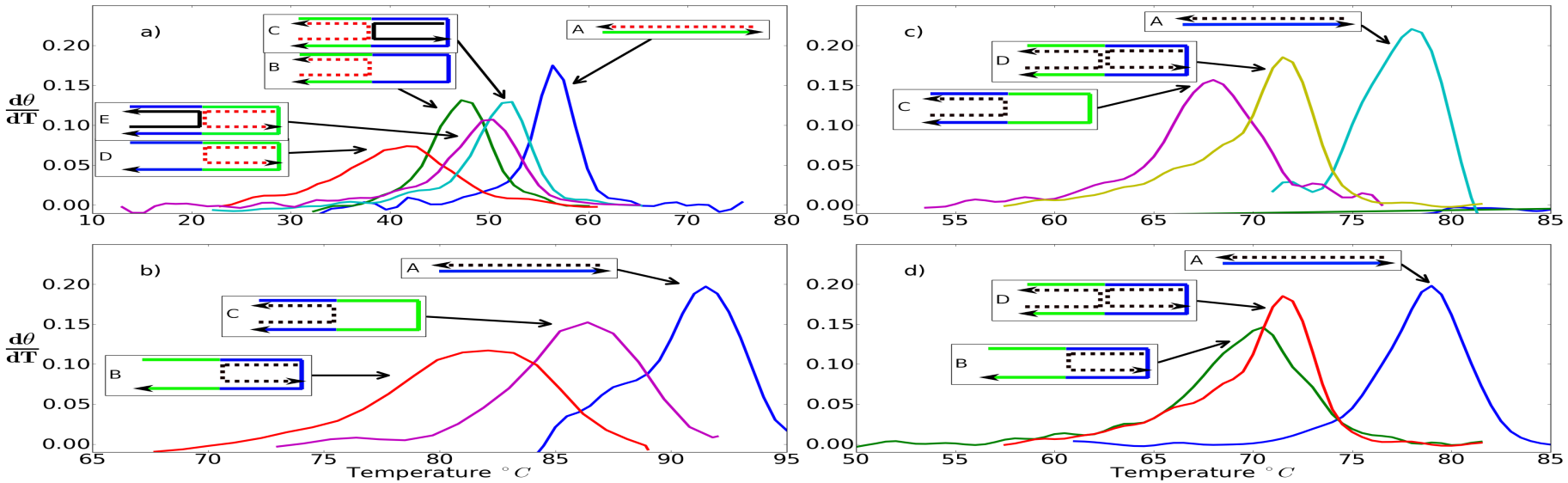


Fig. 3. The derivative $d\theta/dT$ reported in the four figures corresponds to the folding of the dotted staple. a) experimental data on the folding of B1(AT) cases (A,B,D) in the absence of B2(GC), cases (C,E) with B2 already folded; b) experimental data on B2 without B1; c) experimental data on B1m; d) experimental data on B2m

Arbona, J. M., Elezgaray, J., & Aimé, J. P. (2011). Modelling the folding of DNA origami. arXiv preprint arXiv:1111.7130. Retrieved from http://arxiv.org/abs/1111.7130

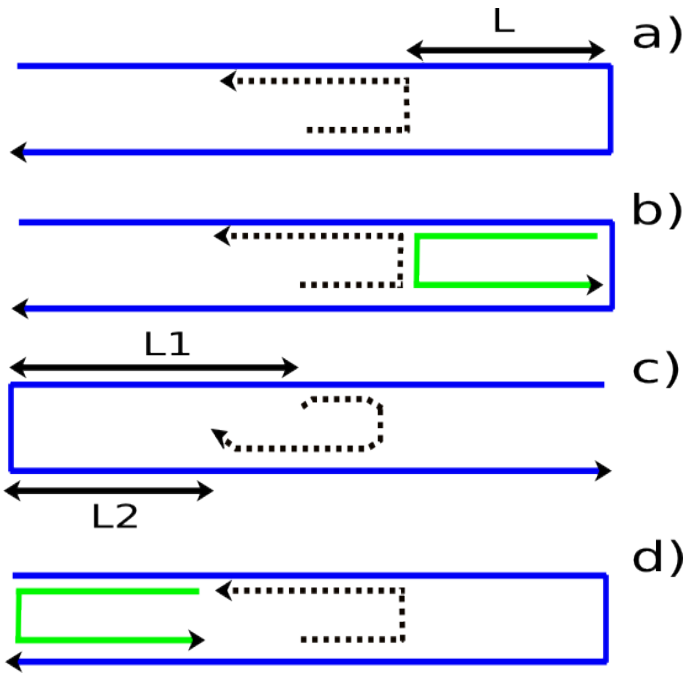


Fig. 4. Computing the entropic penalty for the three different local intermediate states (LIS). The staple to be inserted is represented by the dotted line, the scaffold by the continuous line. (a) LIS1 (b) LIS2 (c) LIS3. Here, we assume that, because of the curvature constraints imposed by this configuration, the staple remains partly unfolded. (d) A typical situation where two types of LIS (LIS1 at the right side of the staple, LIS3 at the left side) can be attributed to a given crossover.

Arbona, J. M., Elezgaray, J., & Aimé, J. P. (2011). Modelling the folding of DNA origami. arXiv preprint arXiv:1111.7130. Retrieved from http://arxiv.org/abs/1111.7130

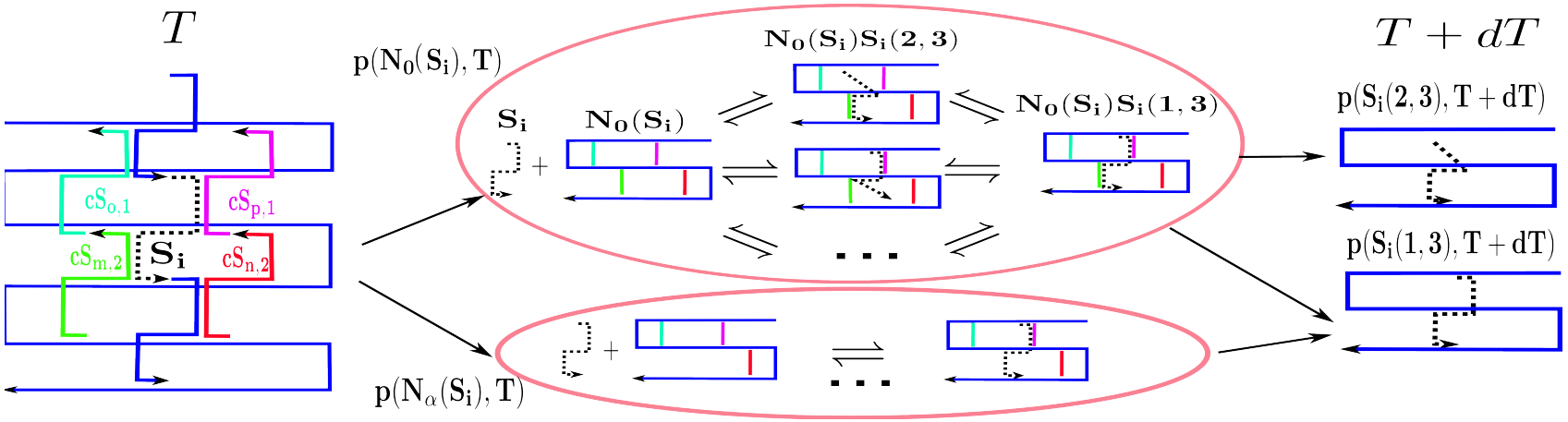


Fig. 5. To evaluate the fraction of the staple S_i folded at the temperature $T + dT$, one considers the nearby staples of the staple i at T and calculates the probability of the different neighbouring crossovers configurations ($cS_{m,2}, cS_{p,1}$,etc) around S_i. The origami is then subdivided in different partially folded state(eg $N_\alpha(S_i)$) with a given probability (eg $p(N_\alpha(S_i))$..). For each of these partial states the equilibrium constant for a partial folded configuration ($N_\alpha(S_i)S_i(m, n)$) of the staple within this restricted local state is calculated as explained in the energy model. The law of mass action for each partial configuration folded gives a set of coupled equations. Once solved they allow to determine the fraction of partial configuration folded in this environment $p(N_\alpha(S_i), T + dT)$. Then we can calculate the total fraction of each configuration folded $p(S_i(m, n), (T + dT)$, as the sum of the fraction of those configurations in the different local states, weighted by the probability of each state.

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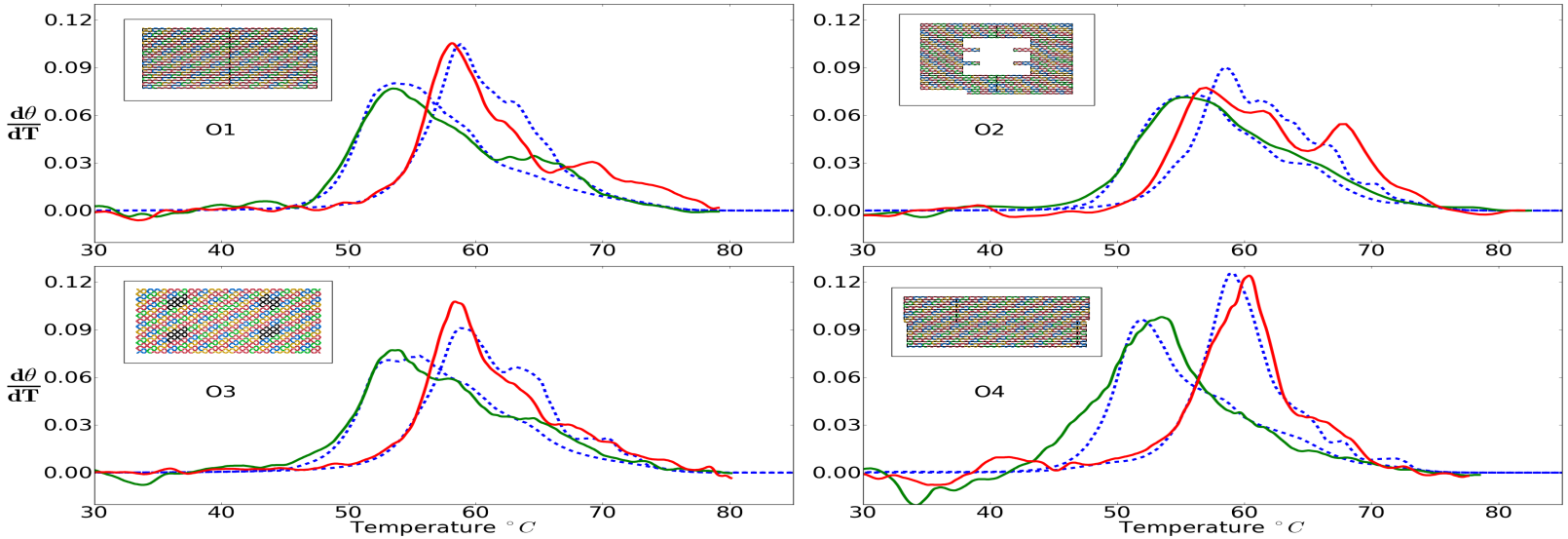

Fig. 6. Derivative of the pairing degree versus temperature. The data corresponding to annealing are in red, melting in green, the model is in blue for both processes.

Arbona, J. M., Elezgaray, J., & Aimé, J. P. (2011). Modelling the folding of DNA origami. arXiv preprint arXiv:1111.7130. Retrieved from http://arxiv.org/abs/1111.7130

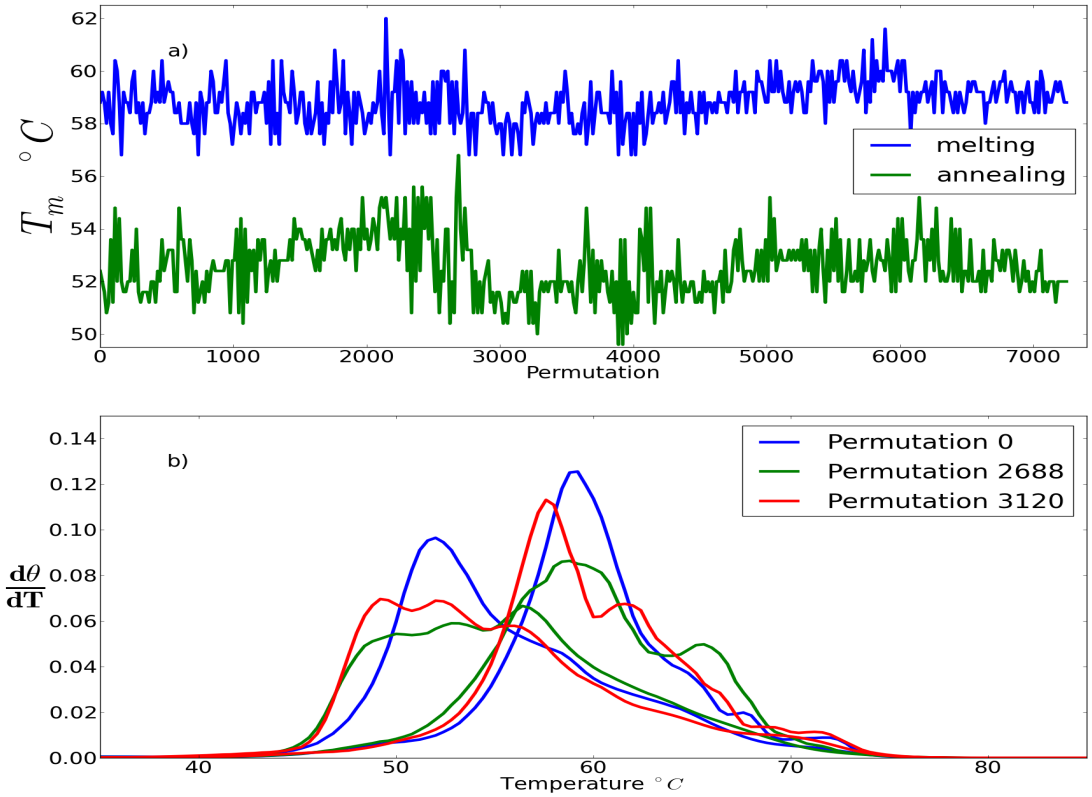

Fig. 7. (a) Distribution of melting temperatures (annealing and melting) as a function of the order of the circular permutation of the scaffold strand. (b) two different melting curves corresponding to two permutations with the lowest and highest annealing temperatures.

Arbona, J. M., Elezgaray, J., & Aimé, J. P. (2011). Modelling the folding of DNA origami. arXiv preprint arXiv:1111.7130. Retrieved from http://arxiv.org/abs/1111.7130

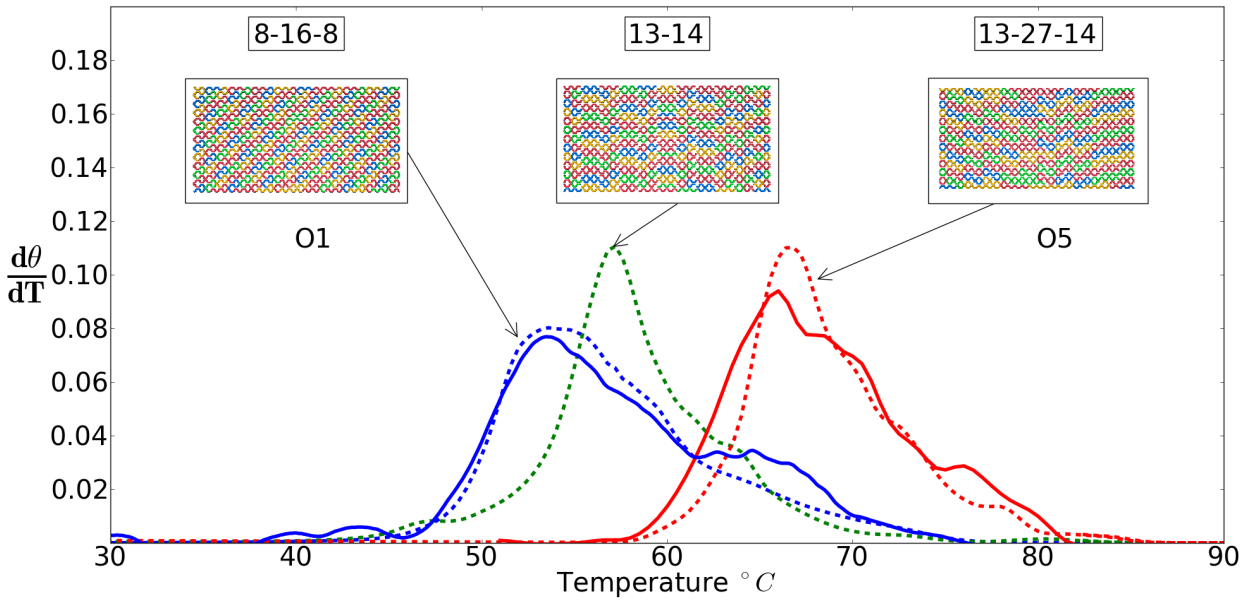

Fig. 8. Annealing curves of the O1 and O5 origamis. The two origamis correspond to the same scaffold pattern, but different staple pattern (solid line = experimental data, dashed line = theoretical curves)

Arbona, J. M., Elezgaray, J., & Aimé, J. P. (2011). Modelling the folding of DNA origami. arXiv preprint arXiv:1111.7130. Retrieved from http://arxiv.org/abs/1111.7130