

Nucleic Acid Tube Grapher (NATuG 3.0)

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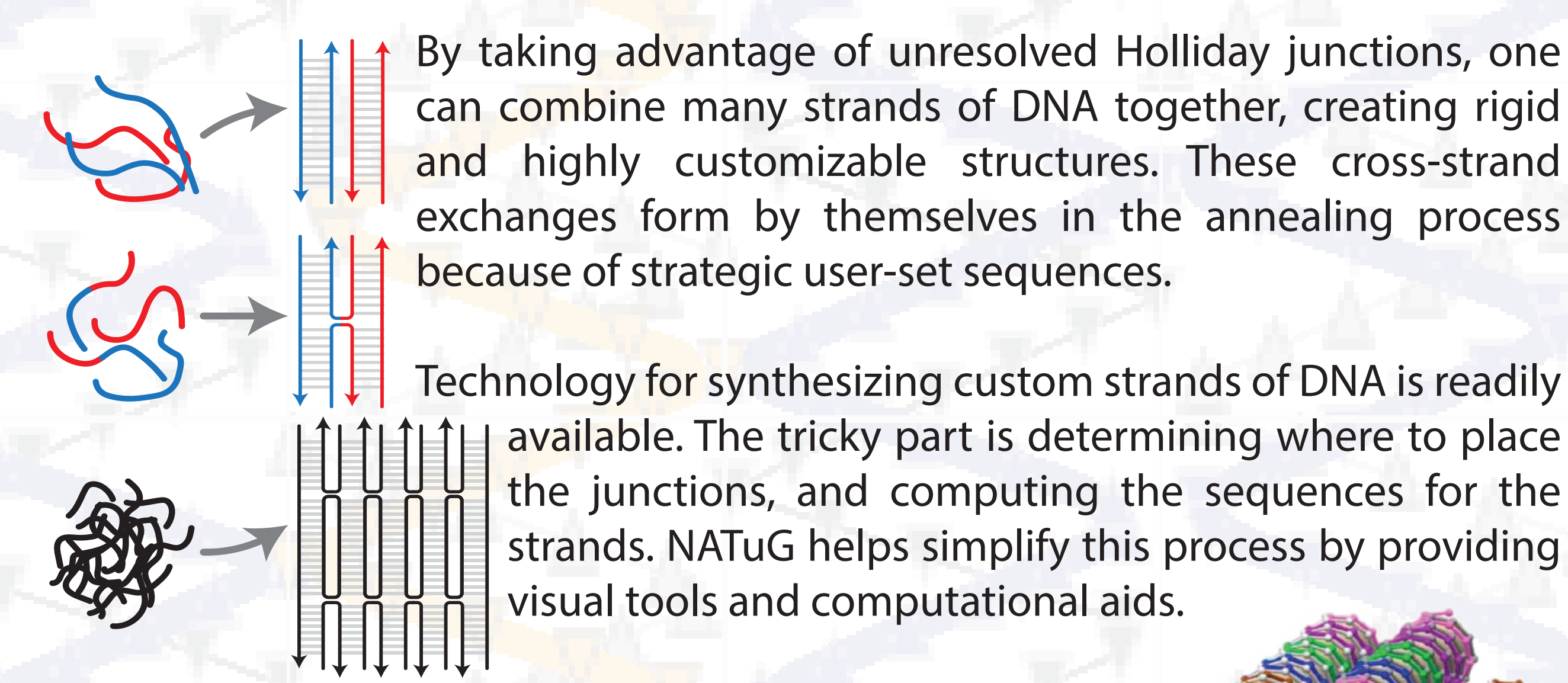
Introduction

Structural DNA Nanotechnology utilizes simple Watson-Crick base pairing to join together different strands of multiple double helices. In this way, one can 'weave' together many helices to create larger, rigid nanostructures. These structures, given that they are made of DNA, possess various unique properties, such as self-assembly, a high level of customizability, and precision of around 1nm.

Designing hollow tubular structures from parallel DNA double helices (a type of DNA nanotube) requires an extensive design process, involving identifying a tube shape that is consistent with the intrinsic geometry of a DNA double helix, aligning helices, placing connections between the helices as to generate minimal strain, and determining base sequences. Additionally, one must determine the number of bases between strand crossovers, and the locations of nicks.

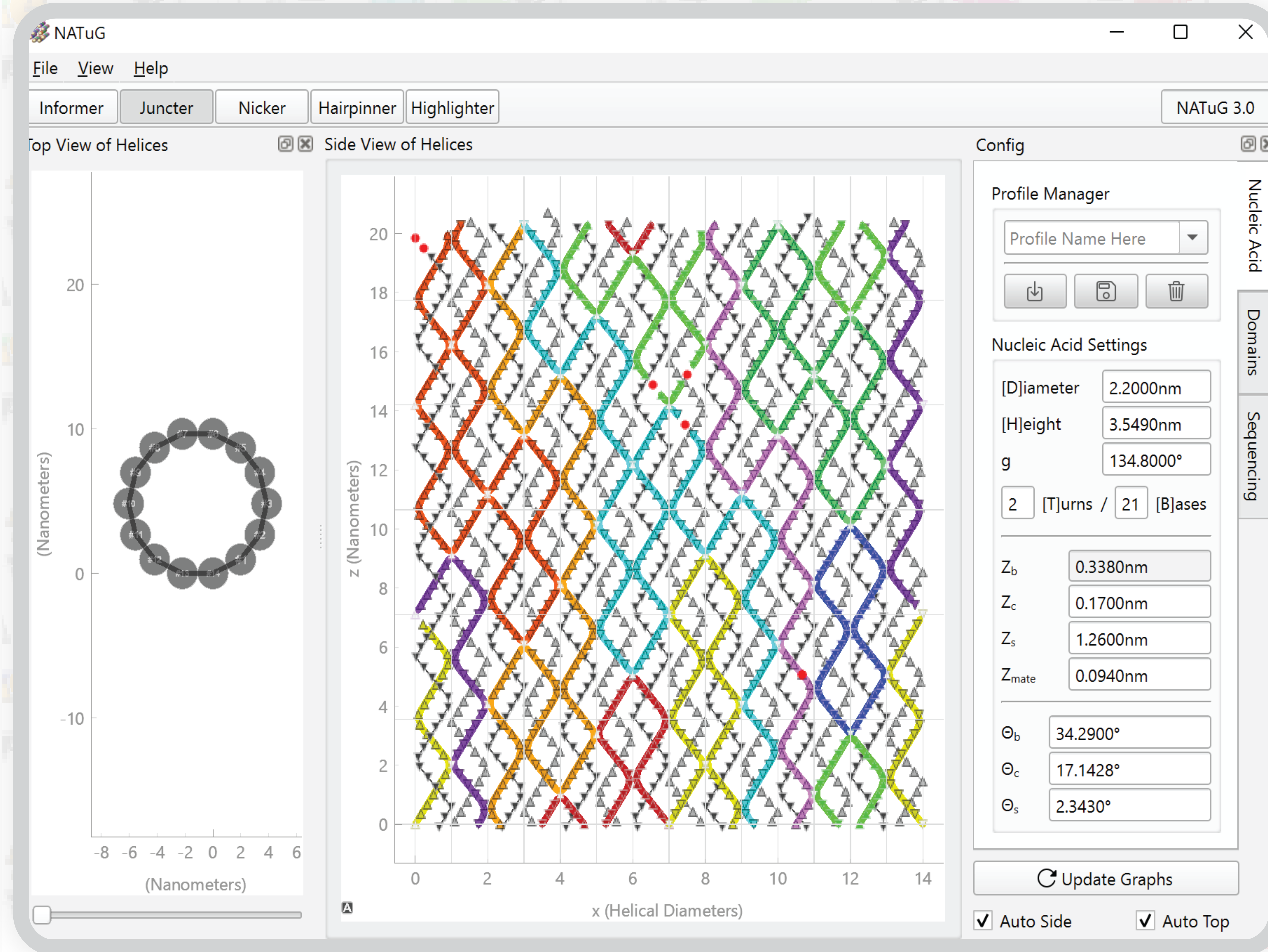
NATuG provides both a powerful interface that helps the user visualize structures in real-time and a robust computational backend that creates manipulatable and exportable data-structure representations of strands. This version of NATuG (3.0) is a ground-up overhaul of previous versions. Unlike prior versions, this version allows for over 14 domains (double helices), can handle junction and nick assignment, and is highly scalable.

Self-Assembly of DNA Nanotubes



To the right is a 3D model of a DNA nanotube. This specific structure weaves together 10 different DNA double helices with junctions. To create a structure like this, careful thought must be put into junction placement, inter-double-helices angles, and sequence.

Figure Credit: (Sherman & Seeman)



NATuG is a Python-based desktop application that streamlines the DNA nanotube design process. The program allows users to easily set the angles between double helices, while computing a top and side view plot in real time. NATuG automatically lines up the double helices so that nucleoside end midpoints (NEMIDs) of different double helices overlap. With a single click on overlapping NEMIDs, NATuG automatically swerves the strands across double helices, creating a cross-strand exchange. By strategically placing junctions throughout the structure, one can transform isolated double helices into a rigid nanotube.

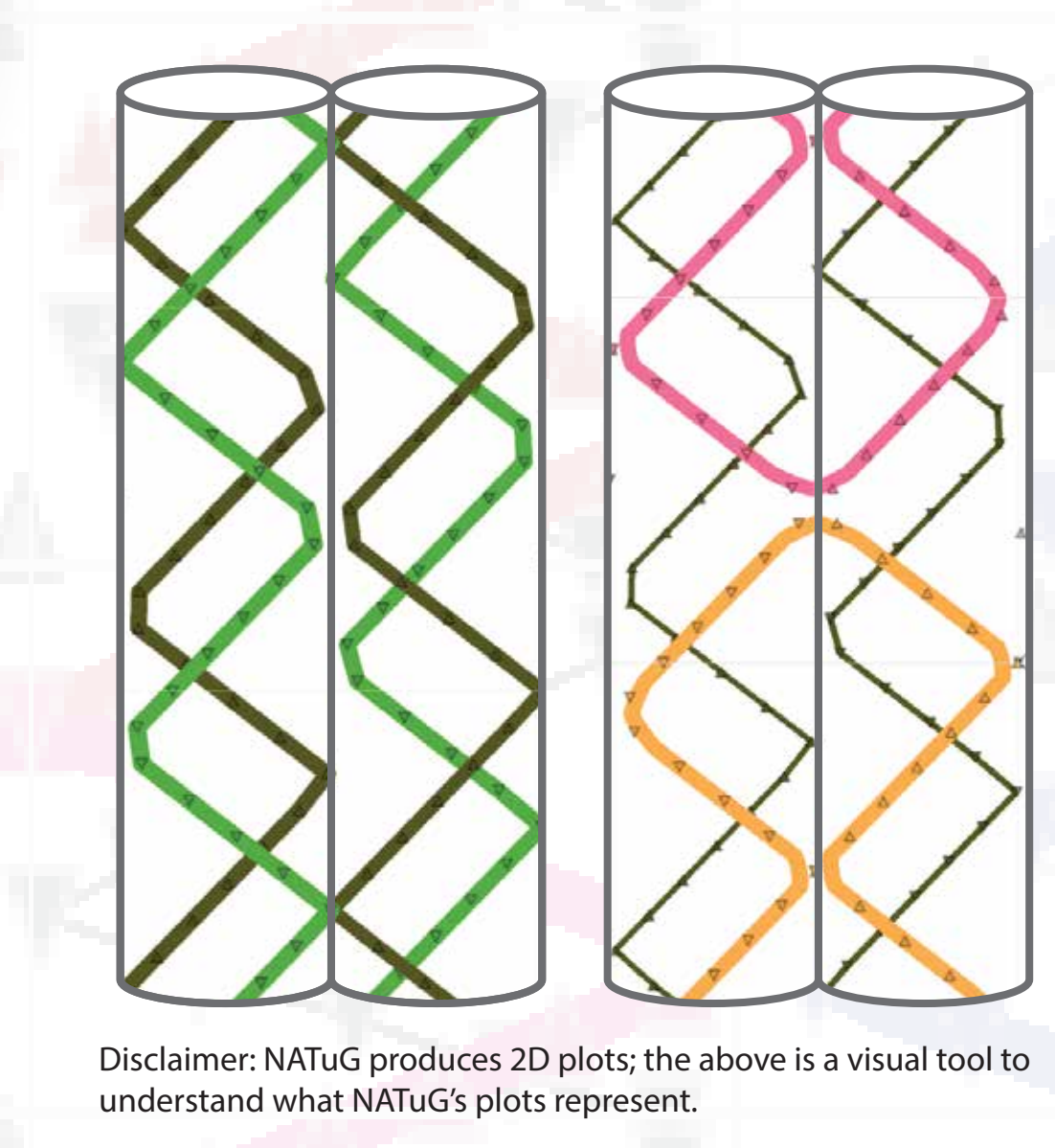
Conclusions

NATuG shows how software can dramatically expedite the DNA nanotube design process and make designing drastically more complex, larger scale nanotubes possible.

Also, although NATuG was initially focused on closed tube design, the program possesses potential for open structures design as well (as can be seen from the example to the right). NATuG's uniquely user-friendly and simple interface allows for rapid design-to-synthesis of basic open structures.

Next Steps

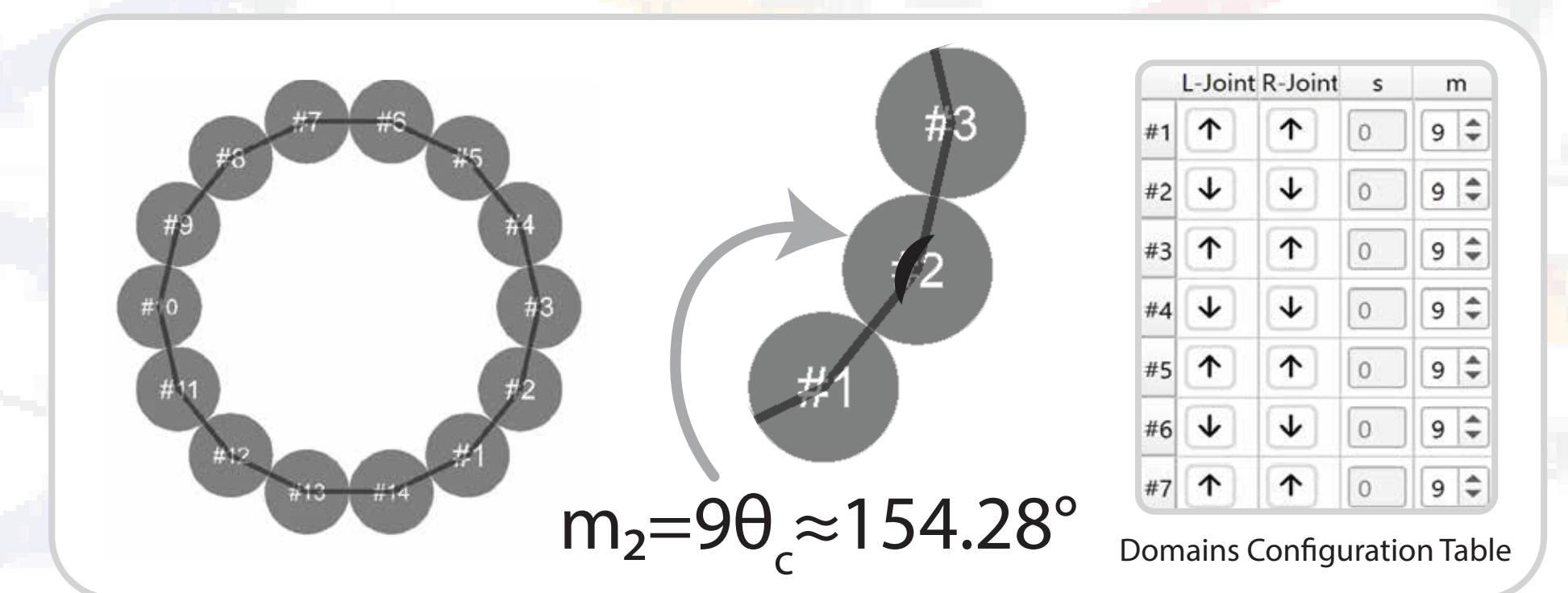
- Sequence Computations**
NATuG currently utilizes randomization to compute sequences. To improve yield rates, we intend to implement standardized methods that reduce sequence repeats and symmetries. Additionally, we would like to contrive additional tools to allow for easier and more interactive sequence editing.
- Enhancing Exporting**
Although NATuG does provide exporting capabilities, the formatting is disparate, and old files quickly stop working as the program updates. The next step will be to create a more comprehensive, optimized, robust, and preview-able export format. We may also implement the ability to export all configurations to a multi-page spreadsheet.
- Linkages/DNA Origami**
Soon, NATuG will also support linkages, which will enable DNA origami based structures. DNA origami involves one long viral strand being folded into a tubular shape by short synthetic "staple strands." This will involve adding a tool to connect the ends of different strands together, and to place additional nucleosides between the ends.
- Additional Design Aids**
In sticking with NATuG's core goal of providing the most user-friendly DNA nanostructure design experience possible, we also hope to add additional design aids. These will range from tools to identify unstably connected domains, to more comprehensive status tips.



To the left are images from NATuG, showing how junctions can 'weave' together multiple helices with just one click. Notice how in the right image the strands swerve across two double helices.

Although these images do not show the interior angle of the double helices, they help demonstrate what NATuG's graph really represents—an 'unrolled' array of double helices. Also, the images showcase how cross-strand-junctions are, as the name implies, strands traversing multiple domains.

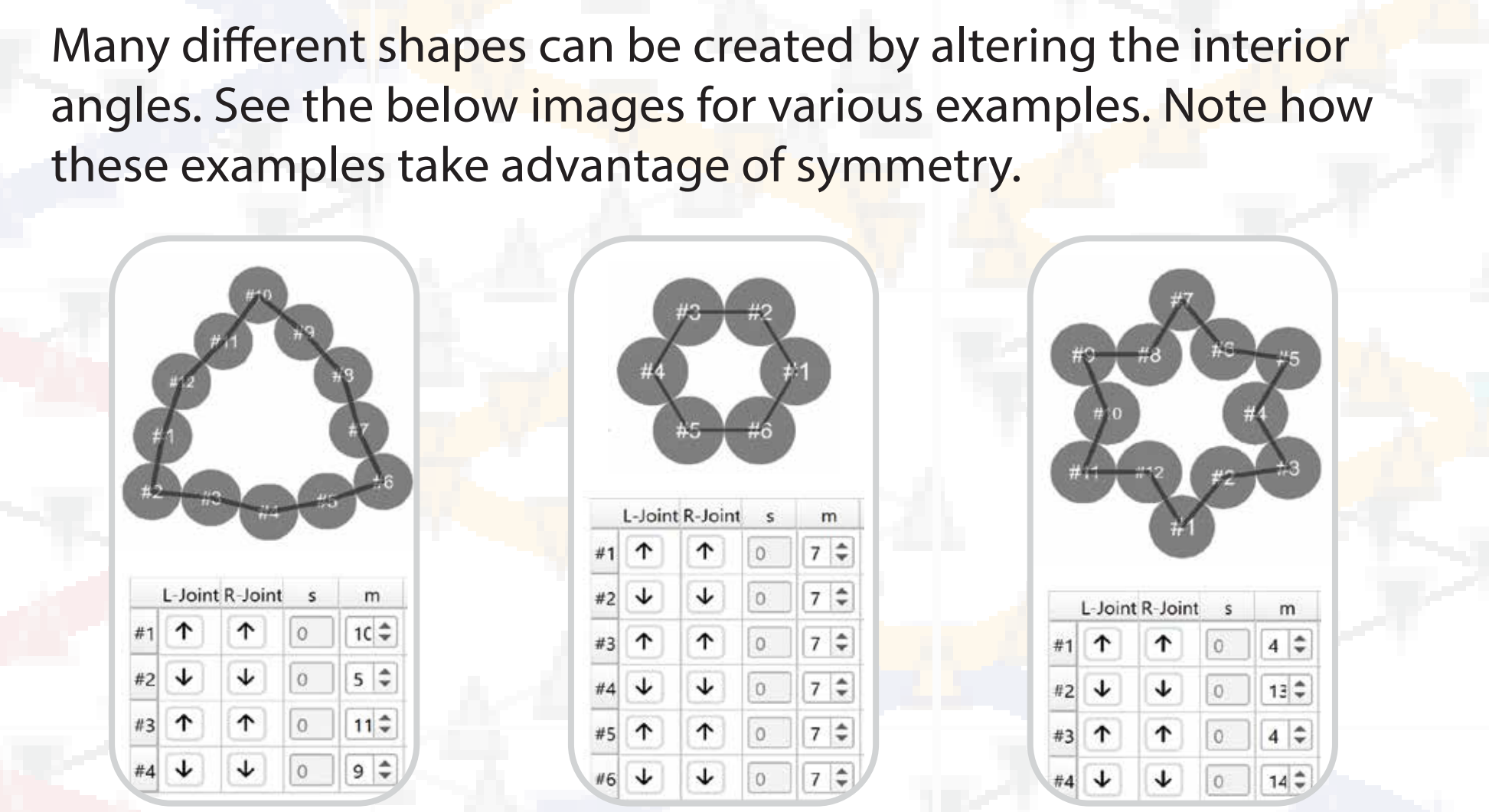
Customizable Tube Shape



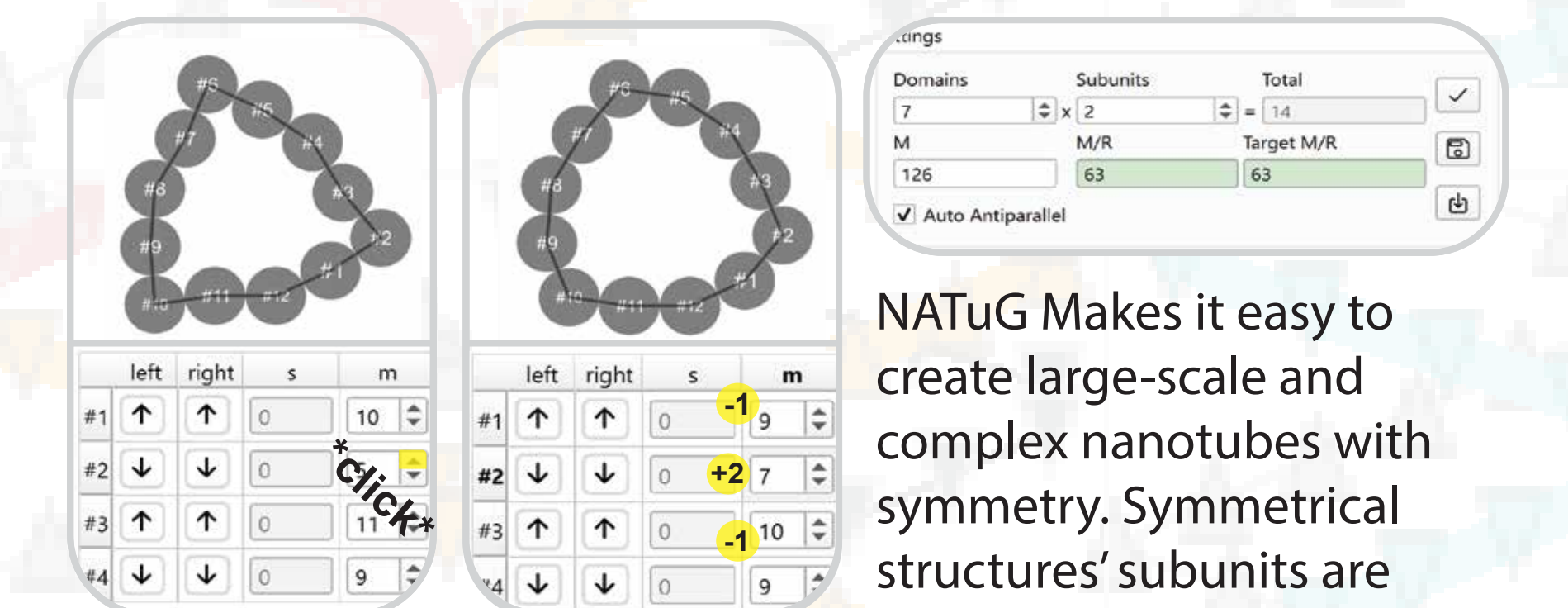
NATuG makes identifying configurations for minimally strained DNA nanotubes convenient and interactive. In the domains configuration table (see top right), NATuG allows for inputs of the interior angle of each domain (double helix). The interior angle, along with strand switches, is what determines the shape of the tube (see top left and middle). As the properties of the domains are changed in the domains table, the top view and side view graphs automatically update in real time. This allows one to see the changes they are making as they make them.

Above one can see a nanotube consisting of 14 double helices, with interior angles of 154.28°. Because the first double helix touches the last double helix (see the large side-view plot in the poster's center), this is a closed tube. However, non-closed structures are possible, and supported by NATuG.

Additional Examples



Symmetrical Design Capabilities



NATuG makes it easy to create large-scale and complex nanotubes with symmetry. Symmetrical structures' subunits are automatically computed, and strands are automatically made anti-parallel.

Plus, NATuG provides useful tools that help users design closed tubes, such as indicators for the total sum of all interior angles for a subunit (top right), and automatic correction when changing an interior angle (top left and top middle).

Domain Exporting and Importing

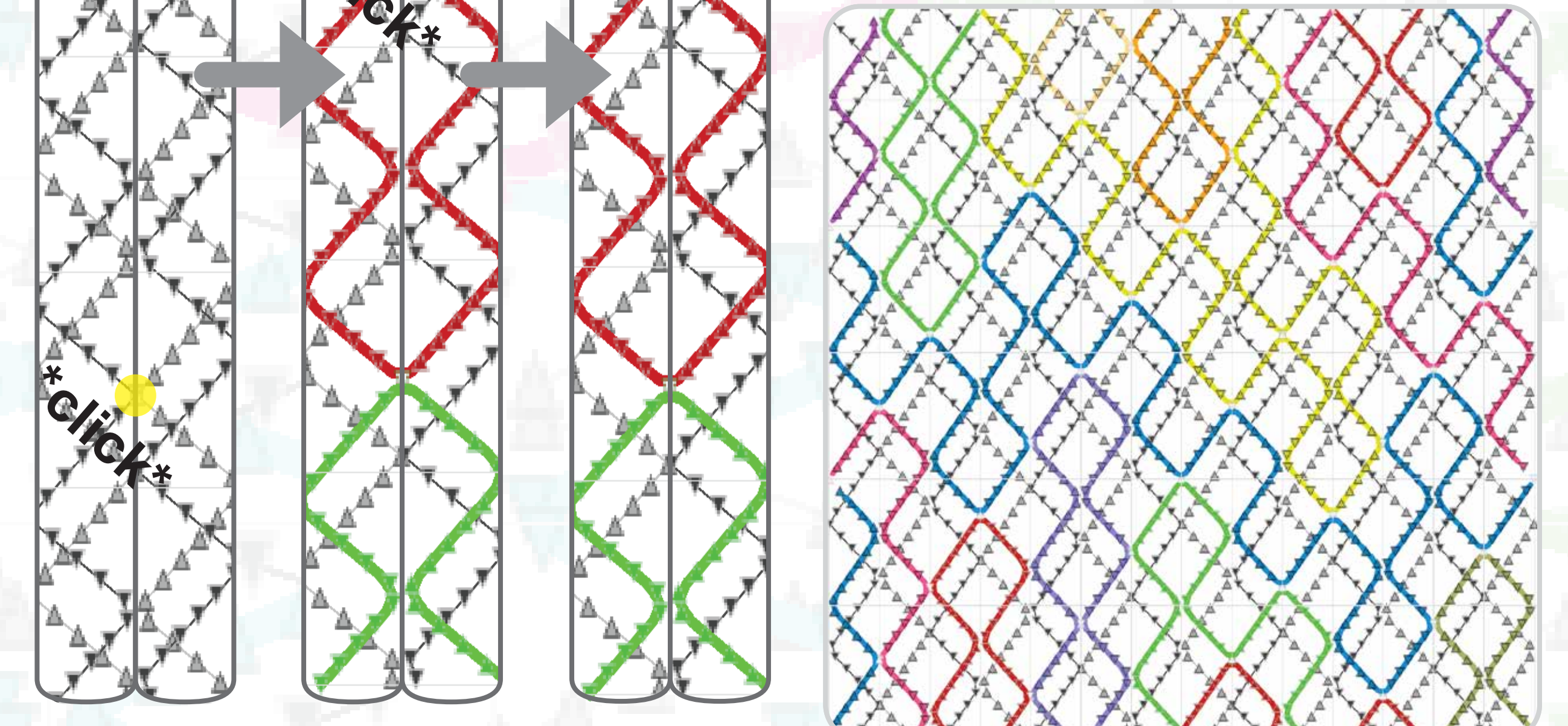
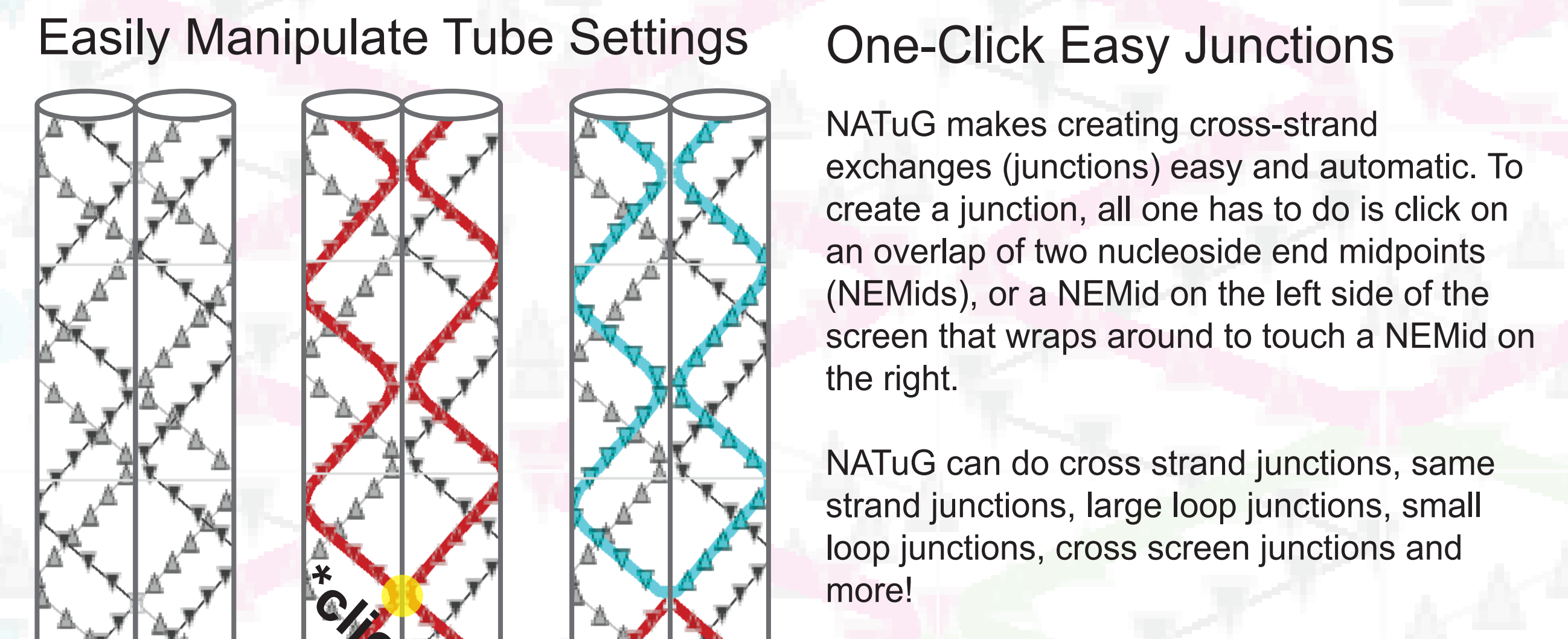
Left Helix Joins	Right Helix Joins	Left Helix Count	Other Helix Count	Symmetry	Antiparallel
9 UP	UP	0-60-0	0-60-0	2 True	
9 DOWN	DOWN	0-60-0	0-60-0		
9 UP	UP	0-60-0	0-60-0		
9 DOWN	DOWN	0-60-0	0-60-0		
9 UP	UP	0-60-0	0-60-0		
9 DOWN	DOWN	0-60-0	0-60-0		
9 UP	UP	0-60-0	0-60-0		

With NATuG, keeping track of your designs is simple. NATuG allows for saving and loading sets of domains—including interior angles, helical joint directions, and symmetry settings—to and from CSV files.

Additional Tools

NATuG also provides additional design tools. As seen above, the top view plot can be rotated, and clicking on specific domains automatically zooms in on the relevant portion of the side view plot.

Automatic Cross-Strand Exchanges



Disclaimer: NATuG produces 2D plots; the cylinder outlines above have been added to help visualize the fact that after creating a junction the strands traverse multiple double helices.

NATuG provides the ability to easily create "nicks" in strands (bottom left). This allows users to split strands, which is particularly useful in preventing strands from tangling during the annealing process. Additionally, nicks open circular strands, which can save time, given the extensiveness of the circular strand synthesis process.

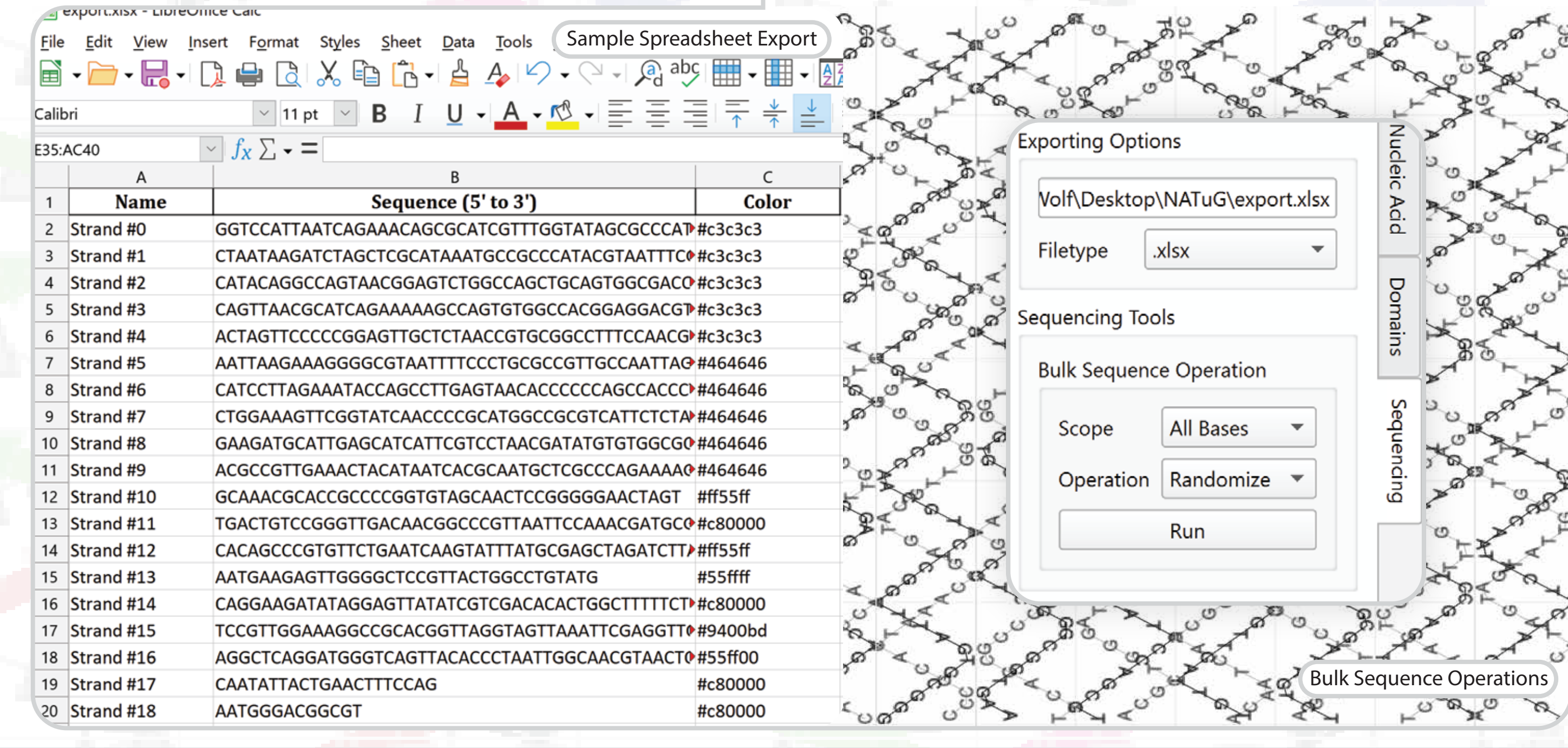
Sequencing Ready

With NATuG, you can click on a strand to see additional information, modify its styles (thickness and color), or alter its sequence.

Within NATuG's sequence-selector dialog there is support for importing sequences from files, randomly generating sequences, and manually entering sequences with a highly user-friendly sequence editor tool. When setting sequences, NATuG automatically sets base complements.

Once all the strand sequences have been set, with the click of a button NATuG can export all the strands to a spreadsheet, with standard formatting, ready to send to labs.

As can be seen in the lower right, NATuG also provides bulk operations that allow for quick assignment of sequences onto all strands.



1) Sherman, W. B.; Seeman, N. C., Design of minimally strained nucleic acid nanotubes. *Biophysical Journal* 2006, 90, (12), 4546-4557.
2) S.M. Douglas, J.J. Chou, W.M. Shih; DNA-nanotube-induced alignment of membrane proteins for NMR structure determination; *Proc Natl Acad Sci U S A*. 2007;104: 6644-6648.
3) Kumara, M. T., Nykpanchuk, D., & Sherman, W. B. (2008). Assembly pathway analysis of DNA nanostructures and the construction of parallel motifs. *Nano letters*, 8(7), 1971-1977.