



A Gamer's Guide to Eterna

Andrew Murphy

A GAMER'S GUIDE TO ETERNA

written and illustrated by Andrew Murphy

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Published by Keystone Research Solutions, LLC, Odenton, Maryland, United States of America.
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Available at <https://www.keystone-research-solutions.com/en/item/a-gamers-guide-to-eterna> .

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First Edition

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PREFACE

Our company, Keystone Research Solutions, offers work internships as a community service to high schools in the Anne Arundel County Public Schools system in Maryland. High school seniors, as interns, can assist a variety of research programs over the Internet with our supervision and mentorship.

Beginning July 2014, we included Eterna (commonly spelled EteRNA at the time) into the mix of research programs our interns could assist. By the end of 2019, intern participation in Eterna had grown enough that it made sense to gather and organize the accumulated experiences, to benefit future interns.

I began work on the guide on New Year's Day 2020. For the first two months, I concentrated on gathering and organizing notes, and in March began writing content in earnest. I took the perspective of writing to a gamer who enjoys solving puzzles and who is just being introduced to Eterna. I wrote through March and April. As Eterna turned its attention toward the COVID-19 pandemic, the guide was taking shape well enough to distribute drafts and to receive feedback from Eterna players and developers. For the past three weeks, I worked on the guide's illustrations.

I hope you enjoy this guide and find it useful. Please [support its production through our Patreon page](#).

~ Andrew Murphy, May 2020.

INTRODUCTION

From a gamer's point of view, Eterna is a way to solve challenging puzzles that have applications in real life.

Eterna is an online community that has a video game at its front end and a biomedical laboratory consortium at its back end. The community centers around a website at <https://eternagame.org>. Its video game presents puzzles to be solved — puzzles representing ribonucleic acid (RNA) sequences we want to fold into desired shapes. Solving the puzzles builds skill at designing RNA sequences. Some of our puzzle solutions might be tested in real life, and some of those might prove useful to biomedical research. Also, some of our puzzle solving techniques might prove useful to developing the state of the art, in game and in real life.

The Eterna game's initial release was in 2010, and generations of terminology (and graphics) have grown with the game. What we call a “chain” here in this guide was often called a “string” or “strand” in forums and other guides. What we call a “misfold diagram” here in this guide has been described elsewhere in a variety of ways — “minimap” was perhaps the most descriptive. If you browse through the volumes written about Eterna, you will certainly notice the casual jargon appearing and changing over time. This is okay: we're a large group of gamers, citizen scientists, and researchers exchanging ideas.

You do not need to understand RNA to play the game and contribute your talent. This guide will help you play with the general gaming skills you already have. It is your gaming skills that the Eterna community seeks. Your creativity is what the community needs.

GAME MECHANICS

GAME PIECES

Imagine a fine jewelry chain that keeps trying to curl and fold upon itself. In fact, you would have to tug on both ends to get the chain to straighten. It wants to curl and fold into a structure influenced by every link in the chain. As a gamer, your basic goal in Eterna is to arrange the links so that the chain folds into the structure you want.

The chain's links are your game pieces, and there are four kinds: A, U, G, and C. Any link in the chain can be any of the four kinds of pieces.



Figure 1. Game pieces A (yellow), U (blue), G (red), and C (green) as used in this guide.¹



Figure 2. Nonspecific game pieces used in this guide for illustrations.

Beyond its immediately linked neighbors, each game piece attracts certain other game pieces. “A” and “U” attract each other. “U” and “G” attract each other more weakly. “G” and “C” attract each other more strongly. We can summarize the game piece attractions like this:

$$A = U - G \equiv C$$



Figure 3. Showing attraction as a little cloud or cotton ball between game pieces in this guide.

¹ Our illustrations here use a metaphor theme, and are not the same as the in-game graphics.

VIEWING MODES

You can view the chain of game pieces in two modes: target mode and natural mode.

In target mode, the chain appears to fold into the structure *you* want to achieve. This is your goal structure. In this viewing mode, you can see where you would likely want to work on game pieces.

In normal mode, the chain appears to fold into the structure *it* wants to achieve. This structure can be quite different than what you wanted. In this viewing mode, changing a single game piece can cause an immediate refolding of the structure.

Target mode shows what you want the chain to do, and natural mode shows what the chain really does in the game. Flipping between target mode and natural mode (like a short [flip book](#) or a [blink comparator](#)) can show you where the natural mode structure “misfolds” — that is, folds differently than what you want.

DIAGRAMS

The Eterna game screen shows you a set of diagrams for the current puzzle, depending on your unlocked capabilities.

The large area in the middle of the screen shows you a diagram of the chain in either target viewing mode or natural viewing mode. You can flip between the two viewing modes. You can zoom and pan the diagram, but not rotate it. This diagram is where you *interact* with the game pieces.

Along the bottom of the screen is a *tool bar* with a variety of icons according to what capabilities you've unlocked so far in the game. The left icons activate a variety of game controls: screenshot, settings, specs, reset, copy, paste, jump and view range; some scripts; and viewing modes. The middle icons control the action of your cursor when you click on a game piece. The right icons control zooming and “undo” or “redo” actions.

You can right click on the game screen for a *pop-up menu* of available actions.

The upper part of the screen has supporting diagrams and information. Here are the current puzzle's title; constraint diagrams and a misfold diagram; and energy information. A help icon is on the right.

The borders of the *constraint diagrams* will generally be red or green. Red signifies you have not met that constraint, and green signifies you have met that constraint. A check mark emphasizes your meeting that constraint.

The *misfold diagram* features a miniature version of the target mode structure, with parts of the structure in white or red. These white and red parts compare the target mode structure with the natural mode structure. White parts show where the natural mode structure folds correctly; and red parts show where the natural mode structure folds incorrectly (that is, where the natural mode “misfolds”). When this miniature structure is all white, there is no difference between the natural mode structure and the target mode structure — which is what you want.

The *energy information* shows “total” energy, “natural/target delta” energy, and the “loop” or “stack” energy if your cursor hovers over the right spots in the large diagram.

KEYBOARD ACTION

There are some keyboard equivalents for tool bar icons and actions. (Hovering your cursor over the tool bar's game piece and pair icons will show a tip that includes the keyboard equivalent.)

1 = Set a piece to A.
2 = Set a piece to U.
3 = Set a piece to G.
4 = Set a piece to C.
5 = Swap a pair.
q = Set a pair to AU.
w = Set a pair to UG.
e = Set a pair to GC.
+ = Zoom in.
- = Zoom out.
z = Undo.
y = Redo.
space bar = Flip viewing mode.
ctrl key = Toggle a game piece black outline marker.
n = Toggle game piece index numbers.
g = Toggle energy display.
, = Toggle game screen background graphics.

Table 1. Keyboard equivalents for some game actions.²

² [“Hotkeys”](#) in the Eterna Wiki has a full list.

GAME SETTINGS AND FEATURES

When you first begin to play Eterna, you will have a basic set of game settings and features. As you gain experience, the game will unlock more capabilities and accesses.

Please note: Eterna changes over time. Some of the things we explain here may be different for you at the time you play.

The Eterna website home page at <https://eternagame.org> has several navigation paths to the puzzles. A path might lead you to a puzzle's description and comment page; a puzzle's "mission" page; a start page for a progression of puzzles; a table of challenge puzzles; a table of player designed puzzles; a lab landing page; a table of lab puzzles; a table of submitted lab designs; or a lab data browser. Some of these pages will have a "play" or "play puzzle" button that leads you to the game screen for a single puzzle.

A game screen has a set of diagrams (see "Diagrams"), a link to "home", an in-game chat side panel, and maybe a toggle button for a "hint".

A tutorial puzzle might start by running an in-game script to take you on a tour around the screen. The puzzle title might link directly to the puzzle's description and comment page.

When you solve one puzzle in a progression, a sidebar will congratulate you and show you a button to the next puzzle.

Occasionally, a pop-up notification will congratulate you on achieving a milestone or unlocking a capability. Your achievement might then appear among the badges on your player profile page. (Click your user name in your browser window's upper right corner to go to your profile page.)

The "screenshot" tool immediately takes a simplified picture of your game screen, and asks whether you want to post the screenshot to the in-game chat channel. The screenshot will include your player name, the puzzle identifier and title, and your viewing mode. You can view the screenshot file and download it, if you want.

BASIC GAME PLAY

The basic goal in an Eterna puzzle is to arrange the game pieces so that their chain folds into the structure you want.

Early game

The first task in solving a puzzle is to identify its specific goal and constraints. In Eterna, the goal may appear on a “mission” page, a puzzle’s description and comment page, or a lab landing page. You could also infer the goal from the constraint and misfold diagrams on the game screen.

In the early game, you will likely identify motifs, partition the structure, and decide an approach. The early game can go swiftly.

Mid game

In the mid game, you will be solving motifs and partitions. This will likely occupy most of your time. Occasionally, you may find that your current approach is not working and that you should return to that step of the early game.

End game

In the end game, you will fulfill remaining puzzle criteria or constraints, maybe having to do with numbers of certain kinds of game pieces, for instance. This could take a little or a long time.

IDENTIFY MOTIFS

In the game screen, a chain will have or will fold itself into a *structure*. Some locations in the structure might show segments of chain lying next to and connected with each other. These locations are where game piece attractions match strongly enough to pull the chain together: these game pieces *pair* with the game pieces directly opposite them. So, we can describe a structure as a set of *paired* and *unpaired* game piece locations.

We call the arrangement of a chain's game pieces a *sequence*. We write the sequence as a series of letters, for example: GUGAAAAAUAC.³

After looking at a handful of puzzle diagrams, we notice common shapes, or *motifs*, recurring within the structures. We can give names to these motifs, and use them to solve the game's puzzles.

Naming and notations

We give index numbers to the game pieces in a chain, starting at "1" and counting to the length of the chain. In this guide's illustrations, piece "1" will always be an end piece with an unattached long stem, and it will usually be on the left end.

We use these index numbers when we write sequences and notations. The numbers help specify what goes where in the chain.

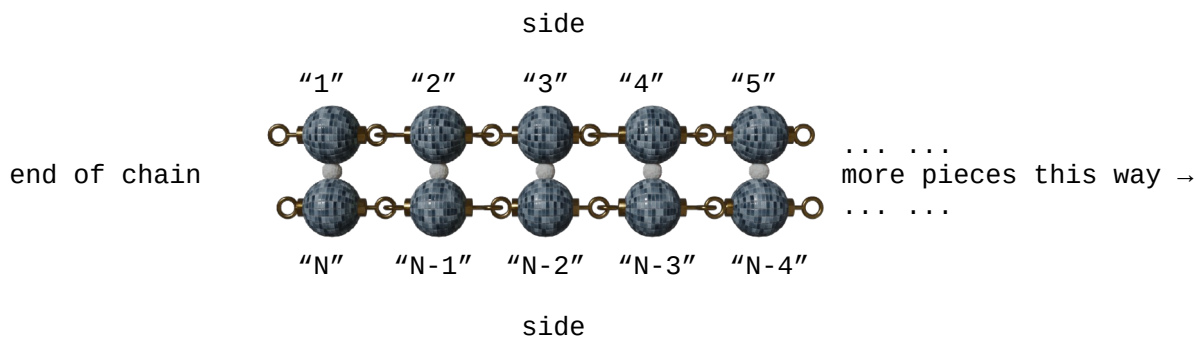


Figure 4. Index numbering. For this chain segment, piece "1" has the unattached long stem at the top left end. Index numbers follow the connected pieces to the right, go on to other parts of the chain not shown, then come back along the bottom row to end at "N". (1, 2, 3, 4, 5, ..., N-4, N-3, N-2, N-1, N)

A label for a specific motif we want to describe consists of the basic type of motif plus the number of game pieces involved in that motif. If there are multiples of this specific motif in a puzzle, we tell each one apart by the index number for its location in the chain.

³ Explanatory puzzle is "[Tutorial 3: Stacks!](#)".

We can describe a structure using *dot-bracket notation*. A dot “.” is an **unpaired game piece** in the chain. A bracket is a **paired game piece** represented by either an open parenthesis “(“ or a close parenthesis “)”. And here in our guide, a hyphen “-” represents a **variable length gap**, a part of the chain that is not part of our discussion at hand.

To find which pieces pair together in the following figure, work from the inside out, matching an open parenthesis on the left with a close parenthesis on the right. If there are multiple “insides”, work outward from each of them to resolve apparent problem areas.

GUGAAAAAUAC ← the sequence
(((.....))) ← the dot-bracket notation, also called the structure notation
 1
123456789012 ← the index numbers

Figure 5. A short example for a chain of length 12.

Working from the inside out in this example, the As from positions 4 through 9 are unpaired. Piece 3G paired with 10U, and we write this pair as GU. Piece 2U paired with 11A, written as UA; and piece 1G paired with 12C, which is GC. We can list the unpaired pieces as simply A,A,A,A,A,A. Starting at index number 1, we can list all the pieces in this chain as GC,UA,GU,A,A,A,A,A,A.

Stack motif

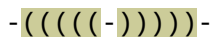
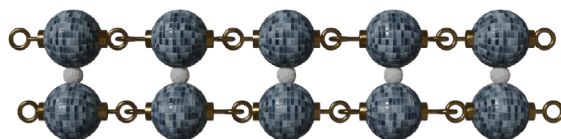


Figure 6. A single long stack, length 5.

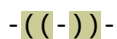
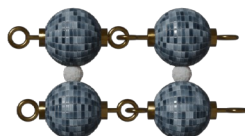


Figure 7. A short stack, length 2.

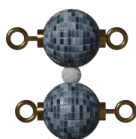
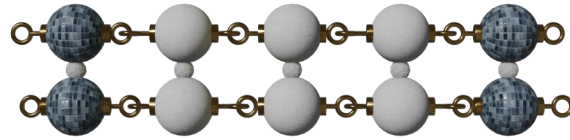


Figure 8. The shortest stack, length 1, also called an isolated pair.

A *stack* (or *stem*) appears wherever there are paired game pieces. The pairs form the stack.

The length of the stack is the number of pairs, not the number of game pieces that are paired. We name a stack by its length: “length 3 stack” or “stack of length 3” are examples. We could also say “3 pair stack”, or simply “3- stack” in shorthand.

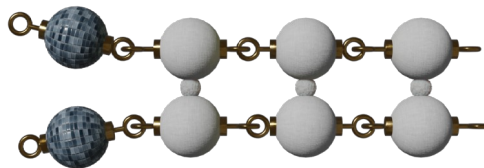
A stack can have a length of 1 or more pairs, but not zero. We consider a stack to be long when it has a length of 4 or 5 or more pairs. A short stack would usually have a length of 2 or 3 pairs, or sometimes 4 pairs if next to a large loop.



-(((((-)))))-

Figure 9. A closing pair at each end of a stack, with each pair highlighted differently.

The last pair at the end of a stack is a “closing pair”. A stack has a closing pair at both of its ends. A stack is ONLY a closing pair if it has length 1, in which case we sometimes call it an “isolated pair”.



-((((-))))-

Figure 10. A terminal mismatch beyond one end of a stack.

Beyond the stack, the first set of unpaired pieces, one game piece on each side, forms a “terminal mismatch”. This means we have gone off the end of the stack, and the game pieces on each side now are a mismatch to each other: they will not form a pair.

Internal loop motif

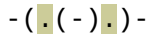
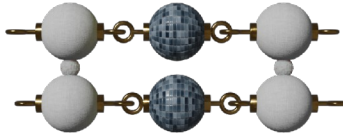


Figure 11. A minimal internal loop between two stacks.

An *internal loop* appears where two stacks meet with unpaired game pieces on both sides of the meeting location. We could also think of this as a location where a single stack splits partially down the middle. It is the unpaired game pieces that are part of the loop itself.

For some purposes, we will include a closing pair while talking about a loop, since the closing pair helps define the loop. Just remember that a closing pair is really part of a stack.

We name an internal loop by the number of unpaired game pieces on each side. For example, we could have a “3-2 internal loop”, or simply “3-2 loop” in shorthand.

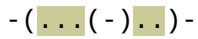


Figure 12. A larger internal loop with uneven sides. This is a 3-2 loop.

Bulge motif

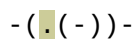
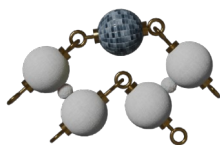


Figure 13. A minimal bulge. This is a 1-0 bulge.

A *bulge* (or *bend*) appears where there are 1 or more unpaired game pieces on one side of a stack but not the other side. We can also think of this as the location where two stacks meet with unpaired pieces only on the outside edge.

We name a bulge by the number of unpaired game pieces on each side, the same way we name internal loops. This way, we can name a minimal bulge as a “1-0 bulge” or more generically as a “1-0 loop”.



Figure 14. A larger bulge. This is a “0-4 bulge” or “0-4 loop”.

Zigzag motif

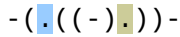
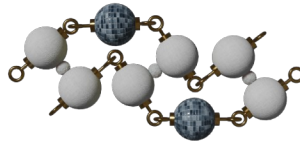


Figure 15. A 1-0,0-1 zigzag using two bulges.

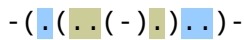
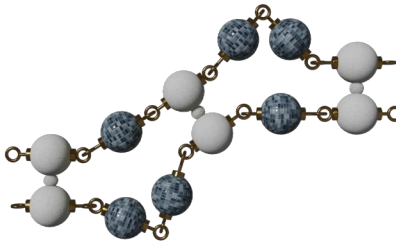


Figure 16. A 1-2,2-1 zigzag using two internal loops.

A zigzag appears as a series of connected bulges or internal loops alternating in sideward directions. The result is that the course of the adjacent stacks appears to shift sideways. If internal loops are involved, each loop will have more unpaired pieces on one side than on the other, to produce the change in direction.

We name a zigzag by the number of unpaired game pieces on each side of each segment. Examples include a “1-0,0-1,1-0 zigzag” and a “1-2,2-1,1-2,2-1 zigzag”.

The outer closing pairs are at each end of a zigzag run. The inner closing pairs appear within the zigzag.

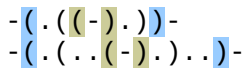


Figure 17. A zigzag's outer closing pairs.

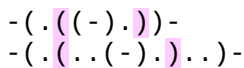
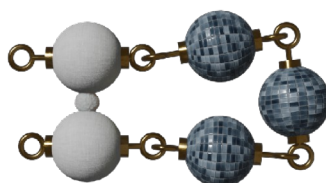


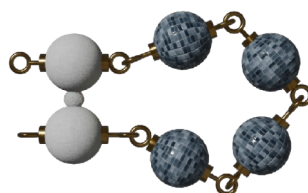
Figure 18. A zigzag's inner closing pairs.

Hairpin loop motif



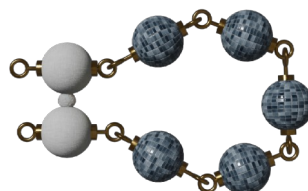
-(...)-

Figure 19. A hairpin loop.



-(....)-

Figure 20. A hairpin loop also known as a tetraloop.



-(.....)-

Figure 21. A hairpin loop also known as a pentaloop.

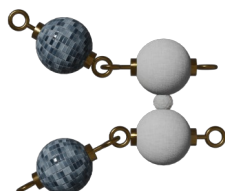
A *hairpin loop* (or *stem loop*) appears where a segment of unpaired game pieces leads from one paired piece around to its partner. By definition, there is only one closing pair associated with a hairpin loop.

We name a hairpin loop by the number of its unpaired game pieces. So, one example could be a “3 piece hairpin loop” or “3- hairpin”⁴; and another example could be a “6 piece loop” or even a “6- loop”.

A hairpin loop name can also use a Greek prefix giving the number of its unpaired pieces: *triloop*, *tetraloop*, *pentaloop*, and so on.

4 To use precise terminology, a “hairpin” is a larger structure (a partition) that includes a set of adjacent loops and stacks, with a “hairpin loop” as the loop motif at that structure’s closed end. We will often be lazy and write just “hairpin” as a shorthand expression for the loop itself.

External loop motif

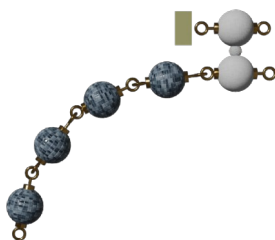


■ (-) ■

Figure 24. A 1-1 external loop.

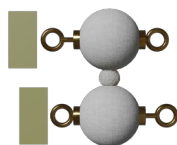
An *external loop* is at the end of the game piece chain.

We name an external loop by the number of unpaired game pieces on each end. An external loop with short pincers could be a “1-1 external loop”. If a multibranch loop is involved, we could have a “2-4-6 external loop”. With pieces dangling off one end only, like a hook, it could be a “0-4 external loop”. This naming can be a bit challenging: if asked, a structure ending in a stack would call its external loop a “0-0 external loop”, even though no loop appears to be there!



■ (-) ■■■■

Figure 25. A 0-4 external loop.



■ (-) ■

Figure 26. A 0-0 external loop!

PARTITION THE STRUCTURE

Look at the structure as a whole. Can you solve it right away, or is it large enough to want to focus on smaller parts of it at a time? If so, look for long stacks and think of them as bridges between loop motifs. Imagine a cut through the middle of each long stack, separating one end from the other. This will partition the structure. The border is not a real thing: it's just your imagination, overlaid onto the structure.

Short stacks generally do not serve as good partition cut locations. Game pieces in the middle of a short stack influence the pieces toward both ends.

If two loop motifs are close enough to influence each other, consider them (and the stack between them) to be members of the same partition. The loops influence each other if changing a game piece in one loop or closing pair causes an energy change for the other loop or closing pair.

Here is a partitioning exercise. Consider the following structure.⁶

```
((..(((.....((((((((((.....(((..(((..((.....))))))....((((.....)))).....
(((((((.....)))))).....))))))....))....
```

Let's identify motifs in the structure.

We immediately see three hairpin loops. The first has 13 unpaired pieces (highlighted in light orange yellow), the second has 8 (very light purple), and the third (very light blue) has 6 pieces.

```
((..(((.....((((((((((.....(((..(((..((.....))))))....((((.....)))).....
(((((((.....)))))).....))))))....))....
```

We also immediately see a 0-8 external loop (highlighted in grayish greenish yellow).

```
(((.....((((((((((.....(((..(((..((.....))))))....((((.....)))).....
(((((((.....)))))).....))))))....))....
```

Looking inward from the external loop at each end of the structure, we see a 2- stack, a 2-2 loop, a 3- stack, a 5-4 loop, and a long 10- stack (gray green). The next groups of parentheses do not match in length (vivid greenish yellow), so we'll stop coming inward for the moment.

```
(((.....((((((((((.....(((..(((..((.....))))))....((((.....)))).....
(((((((.....)))))).....))))))....))....
```

⁶ This is the structure of the "Werewolf-1 predicted structure" puzzle in Eterna.

Now, let's look outward from the hairpins.

From the 6- hairpin (the third hairpin), we count 7 open parentheses to the left and 7 close parentheses to the right — they match, for a stack of length 7 (blue). Further to the right, we run into the 10- stack we have already associated with the external loop.

((..(((.....((((((((((.....(((..(((..(((.....)))))))))..)))....((((.....)))).....
(((((((.....)))))).....)))))).....)).....

From the 8- hairpin (the second hairpin), we count an even set of 4 parentheses to each side, implying a 4- stack (purple). Further to the right, we run into the 7- stack already associated with the 6- hairpin.

((..(((.....((((((((((.....(((..(((..(((.....)))))))))..)))....((((.....)))).....
(((((((.....)))))).....)))))).....)).....

From the first hairpin, a large 13- hairpin, there are two groups of close parentheses (yellow) before running into the 8- hairpin's stack; and there are three groups of open parentheses (yellow) before running into the external loop's 10- stack.

((..(((.....((((((((((.....(((..(((..(((.....)))))))))..)))....((((.....)))).....
(((((((.....)))))).....)))))).....)).....

Looking closer around the 13- hairpin, we first see a single set of 7 close parentheses to the right; and this is matched by a set of 2 open parentheses plus a set of 5 open parentheses to the left (orange). Therefore, there's probably a 1-0 bulge close to the hairpin. We additionally see 3 more parentheses to both the right and the left (orange), implying a 2-2 internal loop and a 3- stack.

((..(((.....((((((((((.....(((..(((..(((.....)))))))))..)))....((((.....)))).....
(((((((.....)))))).....)))))).....)).....

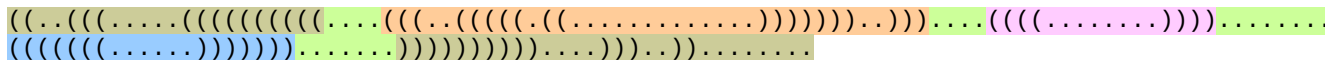
If there are three hairpins and an external loop appearing pretty clearly in the structure, we can infer a multibranch loop might also be present. Indeed, we count 4 unpaired pieces (yellow) between the external loop's 10- stack and the 13- hairpin's 3- stack; 4 more pieces (yellow) between the 13- hairpin's 3- stack and the 8- hairpin's 4- stack; 8 pieces (yellow) between the 8- hairpin's 4- stack and the 6- hairpin's 7- stack; and finally 7 more pieces (yellow) between the 6- hairpin's 7- stack and the external loop's 10- stack.

((..(((.....((((((((((.....(((..(((..(((.....)))))))))..)))....((((.....)))).....
(((((((.....)))))).....)))))).....)).....

This means there is a 4-4-8-7 multibranch loop in the middle of the structure (very light yellowish green).

((..(((.....((((((((((.....(((..(((..(((.....)))))))))..)))....((((.....)))).....
(((((((.....)))))).....)))))).....)).....

Now we can highlight the main areas of the structure.



So, where would we “cut” the structure to make partitions? Let’s bisect long stacks. We cut the long 10- stack (gray green and light green) in half. We cut the 7- stack (blue and light green) into 4+3 or 3+4, either way. We cut the 4- stack (purple and light green) in half. We cut the 5- stack (orange and light green) into 3+2 or 2+3, either way as well. These long stacks bridge the partitions.

The gray green partition fully contains the external loop, a 2-stack, 2-2 loop, 3- stack, and 5-4 loop. The blue partition contains a 6- hairpin. The purple partition contains an 8- hairpin. The orange partition contains a 13- hairpin, 2- stack, and 1-0 bulge. The light green partition contains the 4-4-8-7 multibranch loop, a 3- stack, and a 2-2 loop. Each partition also has a part of the bridging long stacks.

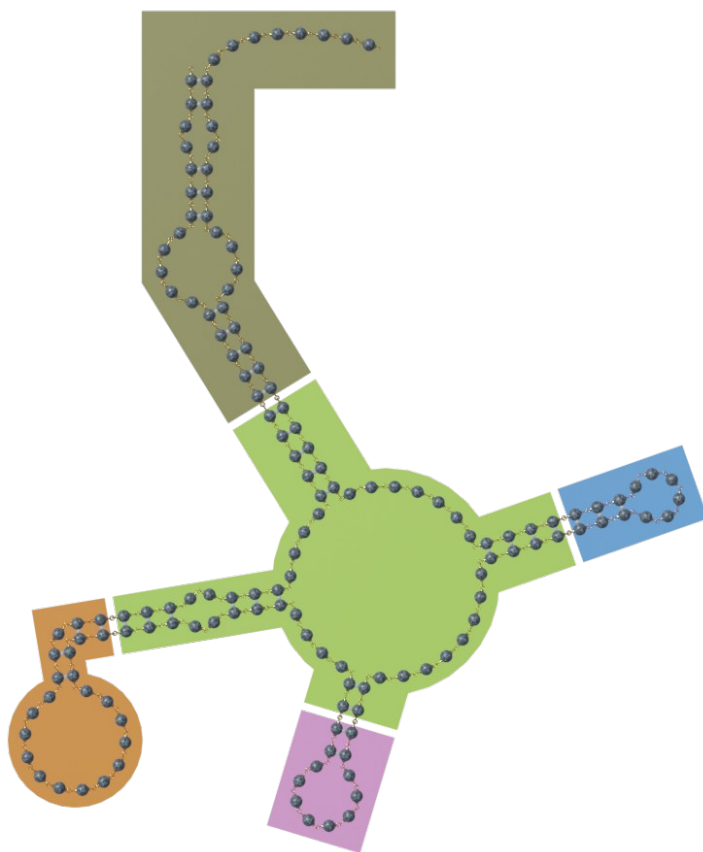
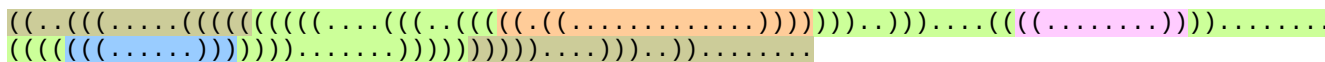


Figure 27. The partitioned structure.

DECIDE AN APPROACH

You could play haphazardly, randomly placing game pieces. Sometimes, this can do something interesting.

Otherwise, try an organized approach to the puzzle. This is where you decide what scenario is involved and what kind of game tactics you want to employ.

For instance, try first to place game pieces along stacks; and then close the loops.

Or, try to close loops first; and then string the stacks with game pieces.

Maybe you want to solve motifs, before fulfilling other puzzle constraints.

Maybe you want to do the “simple” things first, by whatever measure you call them simple.

If your puzzle has a mixture of large and small loops, you might want to approach the large loops first. Eli Fisker suggested⁷ tackling the energetically “pressured” parts of structures, “being hard to stabilize”.

Player drake178 presented⁸ a priority order for solving problem spots. Doing this is equivalent to deciding an approach.

⁷ [in a “Guide addition” comment](#)

⁸ [in the “Measuring up” section](#) of “Advanced puzzle solving guide”

SOLVE MOTIFS

Solve a stack

When solving a stack, we think in terms of game piece pairs. The possible pairs in game are AU, UA, UG, GU, GC, and CG.

Pieces	Sequence
AU, AU, AU	-AAA-UUU-
UG, UG, UG	-UUU-GGG-
GC, GC, GC	-GGG-CCC-
	-(((-)))-

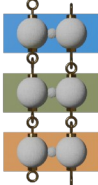


Figure 28. Trying to solve a stack with straight rows of paired game pieces.

With no other factors, we could try to solve a stack with a straight row of paired game pieces. However, game piece pairs behave in a variety of ways when placed together. Here are some handy generalizations. (See the details in the “Swap paired pieces” section.)

- Comparing the pairs against each other, GC and CG are “strong”, AU and UA are “medium”, and UG and GU are “weak”.
- An AU pair can behave differently than a UA pair. Likewise, GC can differ from CG, and UG can differ from GU.
- A GU pair surrounded by AU or GC pairs acts like an AU pair. But, a GU pair surrounded by other GU pairs acts to destabilize those pairs. The same goes for a UG pair.

A common solving technique is to place GC or CG as the closing pair on both ends of a stack, and then use other paired pieces along the middle of the stack.

Another common technique is to alternate a pair along a stack: AU then UA then AU then UA, and so on. We could do the alternations in doublets or triplets, too: “GC,GC” or “CG,CG,CG”.

If there are multiple stacks in the structure, *sequence matching* becomes possible between the stacks. This happens when a side of one stack is attracted more to a complementary side of a different stack. For example, if one stack has four AU pairs in a row, and another stack has three AU pairs in a row, it's possible that an AAA sequence in the second stack could be more attracted to a UUU sequence in the first stack. (Being attracted to the “wrong” stack is a very common cause of a misfold. The chain folds into a structure we don't want — it misfolds.)

We could swap a game piece pair or replace a pair, to prevent such a misfold from happening.

Flipping the game's viewing mode between target mode and natural mode (like a short [flip book](#) or a [blink comparator](#)) can highlight exactly where and how the natural mode structure misfolds. We can prevent or block that particular misfold by swapping a pair or replacing a pair in the offending stack.

Row	Pieces	Sequence	Energy
1	GC, GU, GC	-GGG-CUC-	-1.5 -2.1 = -3.6
2	GC, UG, GC	-GUG-CGC- -(((-)))-	-2.5 -1.4 = -3.9

Figure 29. Length 3 stack examples. Notice how swapping the middle pair affects the energies.⁹

In stacks, we measure energy *between* the pairs. (See “Swap paired pieces” for the details.) We sum these energies to obtain the energy of the whole stack. The more negative the energy, the stronger the stack.

Row	Pieces	Sequence	Energy
1	AU, UA, UA, AU	-AUUA-UAAU-	-1.1 -0.9 -1.3 = -3.3
2	GU, UA, UA, AU	-GUUA-UAAU-	-1.4 -0.9 -1.3 = -3.6
3	AU, UG, GU, UA	-AUGU-AUGU-	-1.4 +0.3 -1.4 = -2.5
4	GU, UA, AU, UG	-GUAU-GUAU-	-1.4 -1.3 -1.4 = -4.1
5	GU, CG, AU, UA	-GCAU-AUGU-	-2.5 -2.1 -1.1 = -5.7
6	GU, CG, GC, UG	-GCGU-GCGU- -((((-))))-	-2.5 -2.4 -2.5 = -7.4

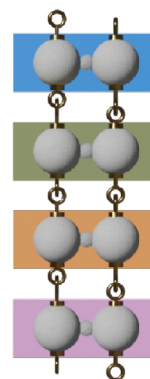


Figure 30. Length 4 stack examples.¹⁰

A suggestion from the Eterna Wiki¹¹ says to start toward a solution by filling in long stacks with AU pairs, and filling in short stacks with GC pairs. The short 3- stacks in the previous figure use GC closing pairs. In the 4- stacks figure above, we compare a variety of arrangements: rows 1 and 2 change a single piece; rows 3 and 4 swap As and Gs; rows 5 and 6 change pairs toward one end of the stack.

9 Examples from “Tips and trick guide” by Eli Fisker in 2011-2016.

10 Examples from “Tips and trick guide” by Eli Fisker in 2011-2016.

11 “Creating a good starting sequence” as of 2013-04-17

One solving technique is to creep a game piece pair along a stack, until we've found a good location for the pair.¹²

Step	Pieces	Sequence
1	CG, UA, AU, GU, UG, A, A, A	CUAGUAAAGUUAG
2	CG, GC , AU, GU, UG, A, A, A	CGAGUAAAGU CG
3	CG, GC , GC , GU, UG, A, A, A	CGGGUAAAGU CCG
4	CG, UA, GC , GU, UG, A, A, A	CUGGUAAAGU CAG
5	CG, UA, GC , GC , UG, A, A, A	CUGGUAAAG CCAG
6	CG, UA, AU, GC , UG, A, A, A	CUAGUAAAG CUAG
7	CG, UA, AU, GC , GC , A, A, A	CUAGGAAAC CUAG ((((((...))))))

Figure 31. Creeping a GC pair along a length 5 stack.

In one variation of the creeping technique, we place a pair where we want to start (step 2 in the figure above); then place the pair at the next location (step 3); then “pick up” the first pair (step 4); and repeat until done. (Step 7 solved the puzzle.)

Step	Pieces	Sequence
1	CG, UA, AU, GU, UG, A, A, A	CUAGUAAAGUUAG
2	CG, CG , AU, GU, UG, A, A, A	CCAGUAAAGU GG
3	CG, UA, CG , GU, UG, A, A, A	CUCGUAAAGU GAG
4	CG, UA, AU, CG , UG, A, A, A	CUACUAAAG GUAG
5	CG, UA, AU, GU, CG , A, A, A	CUAGCAAAG GUAG ((((((...))))))

Figure 32. Creeping a CG pair along a length 5 stack.

In another variation of the creeping technique, we place a pair where we want to start (step 2 in the figure above); then move that pair along, step by step (step 3, step 4, step 5). (Step 5 solved the puzzle.)

¹² Inspired by “The toothpaste method”, a part of Eli Fisker’s “Tips and trick guide”.

Solve an internal loop

When solving an internal loop, or any loop for that matter, we focus on the closing pair and the first unpaired piece on each side into the loop — the terminal mismatch. These pieces affect loop energy.

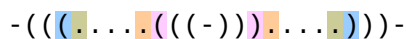
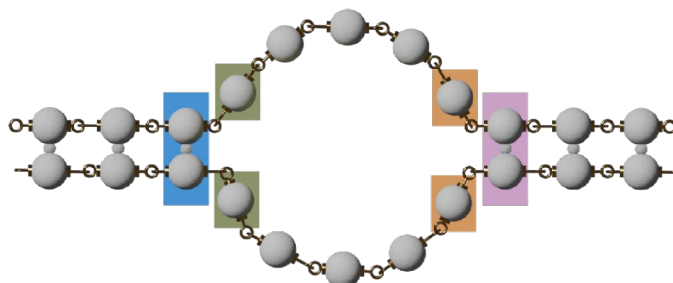


Figure 33. Closing pair and terminal mismatch positions for a 5-5 internal loop.

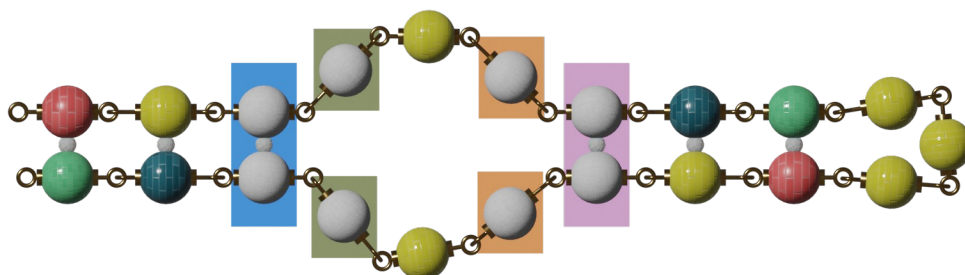
The immediate goal, of course, is to close the loop. The choice of closing pair affects the energy of the loop. The choice of terminal mismatch pieces can affect the loop energy, as well; and the size of the loop determines whether some terminal mismatch pieces have an effect, or not.

We could start with a strong pair, like GC or CG, as a closing pair. If this succeeds in closing the loop, then we can experiment with a weaker pair, to see if it can also do the job.

If a pair isn't strong enough to close the loop, we could "Boost a loop".

Some players prefer to boost the loop first, as a preparatory step, and then they work on the closing pairs. Player Brouard, for instance, noted¹³ that boosting loops before doing other steps prevents some of the lag while solving very large puzzles.

13 on a slide in "Beginner's Guide to Puzzle Solving"



Row	Pieces	Sequence	Loop energy !
1	GC, AU, UA, A, A, A, AU, UA, CG, A, A, A, {CG}, {UA}, {AU}, A, A, A	GAUAAAUCAAAGAUAAAUC	+3.4
2	GC, AU, UA, U, A, U, AU, UA, CG, A, A, A, {CG}, {UA}, {AU}, U, A, U	GAUUUAUCAAAAGAUUAUC	+2.0
3	GC, AU, UA, G, A, G, AU, UA, CG, A, A, A, {CG}, {UA}, {AU}, A, A, A	GAUGAGAUCAAAGAUAAAUC	+1.2 !
4	GC, AU, GC, A, A, A, GC, UA, CG, A, A, A, {CG}, {UA}, {GC}, A, A, A	GAGAAAGUCAAAAGACAAACUC	+2.0 !
5	GC, AU, GC, A, A, A, CG, UA, CG, A, A, A, {CG}, {UA}, {CG}, A, A, A	GAGAAACUCAAAAGAGAAACUC	+2.0 !
6	GC, AU, CG, A, A, A, CG, UA, CG, A, A, A, {CG}, {UA}, {CG}, A, A, A	GACAAACUCAAAAGAGAAAAGUC	+2.0 !
7	GC, AU, CG, A, A, A, GC, UA, CG, A, A, A, {CG}, {UA}, {GC}, A, A, A	GACAAAGUCAAAAGACAAAGUC (((...(((...))))...))) 1 2 123456789012345678901	+2.0 !

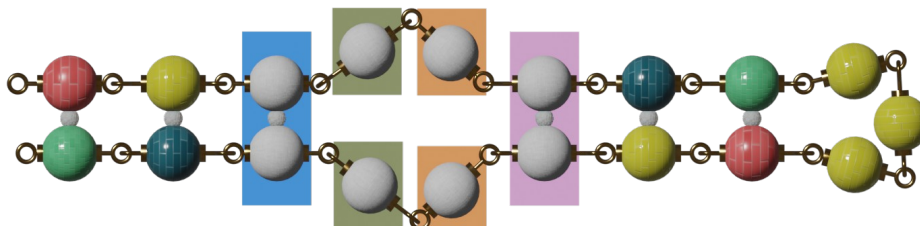
Figure 34. Solving a 3-3 internal loop.

In the figure above, we start with a nearly solved puzzle. The structure has a 3- stack, 3-3 internal loop, 3- stack, and 3- hairpin loop. The stacks and hairpin loop sum to a -1.3 energy, but the internal loop energy is +3.4 (see row 1), giving a total energy of +2.1. We need the internal loop energy to reach +1.3 or lower.

We experiment first with U in terminal mismatch positions, finding in row 2 a sequence that lowers internal loop energy to +2.0. We then try G in terminal mismatch positions. If we start with a G at position 18, a second G in one of the other end's terminal mismatch positions (6, 16) brings the loop energy to +1.2 (yay!), but they cause a misfold in one direction or another. If instead we put the first G at position 4, the second G at either 6 or 16 succeeds in solving the puzzle. (See row 3.) Lastly, we'll note that using C in the terminal mismatch positions has no effect at all on the internal loop energy.

Next, we experiment with closing pairs. Swapping the starting UA and AU pairs (row 1 again) has no effect on loop energy, and neither does replacing them with UG and GU pairs. Putting in GC and CG pairs, though, solves the puzzle in several combinations. GC,GC closing pairs take the internal loop energy to +2.0 and the total energy to -1.4. (See row 4. Note that the closing pairs change stack energies as well as the internal loop energy.) GC,CG pairs yield a +2.0 internal loop energy and a -1.3 total energy (row 5). CG,CG (row 6) does the same as GC,GC (row 4). CG,GC (row 7) leads the pack with a +2.0 internal loop energy and a -1.5 total energy.

You may have noticed that we don't show any results from experimenting with positions 5 or 17 in the 3-3 internal loop structure, above. Positions 5 and 17 are in the internal loop, but are not in the terminal mismatch positions. We did do experiments with positions 5 and 17, and found that they had no loop energy effect at all. Also, experiments with a 4-4 internal loop and some larger loops showed no loop energy effect from such "middle" positions of the internal loop.



Row	Pieces	Sequence	Loop energy !
1	GC, AU, UA, A, A, AU, UA, CG, A, A, A, {CG}, {UA}, {AU}, A, A	GAUAAAUCAAAAGAUAAAUC	+2.8
2	GC, AU, UA, U, A, AU, UA, CG, A, A, A, {CG}, {UA}, {AU}, A, A	GAUUAAUCAAAAGAUAAAUC	+2.0
3	GC, AU, UA, U, A, AU, UA, CG, A, A, A, {CG}, {UA}, {AU}, A, U	GAUUAAUCAAAAGAUUAUUC	+2.9
4	GC, AU, UA, U, U, AU, UA, CG, A, A, A, {CG}, {UA}, {AU}, A, U	GAUUUAUCAAAAGAUUAUUC	+2.0
5	GC, AU, UA, U, U, AU, UA, CG, A, A, A, {CG}, {UA}, {AU}, U, U	GAUUUAUCAAAAGAUUUUUC	+1.1 !
6	GC, AU, UA, A, A, AU, UA, CG, A, A, A, {CG}, {UA}, {AU}, A, A	GAUAAAUCAAAAGAUAAAUC	+2.8
7	GC, AU, UA, A, A, AU, UA, CG, A, A, A, {CG}, {UA}, {AU}, A, G	GAUAAAUCAAAAGAUAGAUC	+1.7
8	GC, AU, UA, A, A, AU, UA, CG, A, A, A, {CG}, {UA}, {AU}, A, G	GAUAAAUCAAAAGAUAGAUC	+0.7
9	GC, AU, UA, A, A, AU, UA, CG, A, A, A, {CG}, {UA}, {AU}, G, G	GAUAAAUCAAAAGAUUGAUC	+0.7 !
10	GC, AU, UA, G, A, AU, UA, CG, A, A, A, {CG}, {UA}, {AU}, G, A	GAUGAAUCAAAAGAUAAAUC	+0.7 !
11	GC, AU, UA, A, A, AU, UA, CG, A, A, A, {CG}, {UA}, {AU}, C, A	GAUAAAUCAAAAGAUCAAUC	+2.3
12	GC, AU, UA, C, A, AU, UA, CG, A, A, A, {CG}, {UA}, {AU}, C, A	GAUCAAUCAAAGAUCAAUC	+1.9
13	GC, AU, GC, A, A, AU, UA, CG, A, A, A, {CG}, {UA}, {AU}, A, A	GAGAAAUCAAAAGAUAAUC	+2.1
14	GC, AU, UA, A, A, GC, UA, CG, A, A, A, {CG}, {UA}, {GC}, A, A	GAUAAGUCAAAAGACAAAUC	+2.0
15	GC, AU, CG, A, A, AU, UA, CG, A, A, A, {CG}, {UA}, {AU}, A, A	GACAAAUCAAAAGAUAAUC (((...(((...)))...))) 1 1234567890123456789	+2.0 !

Figure 35. Solving a 2-2 internal loop.

Let's try the same experiments with a 2-2 internal loop. The structure has a 3- stack, 2-2 internal loop, 3- stack, and 3- hairpin loop. The stacks and hairpin loop sum to a -1.3 energy, but the internal loop energy is +2.8 (see row 1), giving a total energy of +1.5. We need the internal loop energy to reach +1.3 or lower.

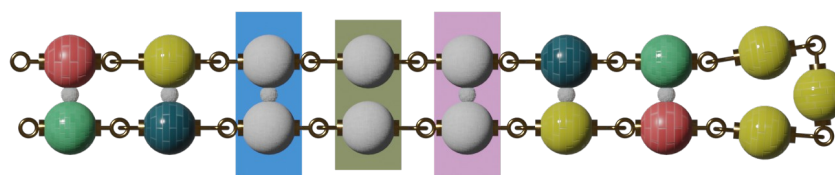
Trying U first, we find that a single U in any of the four terminal mismatch positions (4, 5, 15, 16) will bring the internal loop energy down to +2.0 (row 2). Placing a second U in positions 5 or 15 has no further effect, but placing it in 16 raises the internal loop energy to +2.9 (row 3). Adding a third U at position 5 returns the internal loop energy to +2.0 (row 4). Placing a fourth U at position 15, thus filling

all the terminal mismatch locations with Us (row 5), reduces the internal loop energy to +1.1 and solves the puzzle. Looking at the natural viewing mode along the way, we see that the initial sequence (row 1) does not fold at all. When there is one U in a terminal mismatch position (row 2), that U pairs with the A in the partner mismatch position. Putting a second U in that partner position (row 3) blocks the pairing and prevents folding at all. When we add the third U, in an opposite terminal mismatch position (row 4), that third U pairs with the A in its partner mismatch position. Adding the fourth U (row 5) blocks that pairing, allowing a fold into our desired structure and solving the puzzle.

Next, we experiment with G. Placing a G at any of the terminal mismatch positions (4, 5, 15, 16) lowers the internal loop energy to +1.7 (row 7), but does not allow the chain to fold. If we place Gs at 16 and 5, the internal loop energy decreases to +0.7 (row 8), but the chain misfolds. Placing the two Gs at 16 and 15 (row 9), or at 4 and 15 (row 10), prevents the misfold and solves the puzzle.

Next, we experiment with C. Placing a C at positions 4 or 15 lowers the internal loop energy to +2.3 (row 11), and does not help the chain to fold. Placing that C at 5 or 16 does nothing. Placing Cs at both 4 and 15 lowers internal loop energy to +1.9 (row 12) and causes the chain to misfold to form a GC,AU,UA,CG stack. Placing a third C at 16 raises the loop energy to +2.3 again, but allows the same misfold. With Cs in all four terminal mismatch positions, the internal loop energy returns to its original +2.8, but the chain does the same misfold.

Let's turn our attention to closing pairs. We started with a UA and an AU. If we swap them into an AU and UA, the internal loop energy does not change. Likewise, any combination of UG and GU pairs does not change the internal loop energy. However, the GC and CG pairs do change the internal loop energy. Putting a GC in the 3,17 positions (row 13) reduces internal loop energy to +2.1 and the total energy to -0.2, but causes a misfold. Putting the GC pair instead at the other closing pair position (row 14) takes internal loop energy to +2.0 and total energy to -0.4, but causes a different misfold. Putting a CG pair at the 3,17 positions (row 15) lowers loop energy to +2.0, lowers total energy to -0.4, and solves the puzzle.



Row	Pieces	Sequence	Loop energy !
1	GC, AU, UA, A, AU, UA, CG, A, A, A, {CG}, {UA}, {AU}, A	GAUAAUCAAAAGAUAAUC	+1.7
2	GC, AU, UA, U, AU, UA, CG, A, A, A, {CG}, {UA}, {AU}, U	GAUUUAUCAAAAGAUUAUC	+1.8
3	GC, AU, UA, G, AU, UA, CG, A, A, A, {CG}, {UA}, {AU}, G	GAUGAUCAAAAGAUGAUC	-0.4
4	GC, AU, UG, A, GU, UA, CG, A, A, A, {CG}, {UA}, {GU}, A	GAUAGUCAAAAGAUAGUC	+1.7 !
5	GC, AU, GC, A, AU, UA, CG, A, A, A, {CG}, {UA}, {AU}, A	GAGAAUCAAAAGAUACUC	+1.1 !
6	GC, AU, CG, A, AU, UA, CG, A, A, A, {CG}, {UA}, {AU}, A	GACAAUCAAAAGAUAGUC	+1.1 !
7	GC, AU, GC, A, AU, UA, CG, A, A, A, {CG}, {UA}, {AU}, A	GAGAAUCAAAAGAUACUC	+1.1 !
8	GC, AU, CG, A, AU, UA, CG, A, A, A, {CG}, {UA}, {AU}, A	GACAAUCAAAAGAUAGUC	+1.1 !

(((.((((...))).)))
1
12345678901234567

Figure 36. Solving a 1-1 internal loop.

Let's explore a 1-1 internal loop. The structure has a 3- stack, 1-1 internal loop, 3- stack, and 3- hairpin loop. The stacks and hairpin loop sum to a -1.3 energy, but the internal loop energy is +1.7 (see row 1), giving a total energy of +0.4. We again need the internal loop energy to reach +1.3 or lower.

The 1-1 internal loop is distinctive, in that one set of terminal mismatch positions (4 and 14 in the figure) serves both closing pairs.

We cannot succeed with a single U in the internal loop, because in either terminal mismatch position it would pair with the A in the other position to collapse the loop and form a continuous stack. We could block that collapse by putting a U in both terminal mismatch positions, but in this case that doesn't help (row 2).

Placing a G in either 4 or 16 does not change the internal loop energy. Placing a G in both 4 and 16 lowers the internal loop energy to -0.4 (row 3) and the total energy to -1.7, but causes a misfold by pairing with the Cs in the chain.

Placing a C in either 4 or 16 does not change the internal loop energy. Placing a C in both 4 and 16 also doesn't change the energy.

Experimenting with the closing pairs, now, we find right away that swapping the UA (positions 3,15) and AU (5,13) closing pairs, in any combination, changes stack energies but not the internal loop energy.

Placing a UG pair and a GU pair solves the puzzle (row 4) without changing loop energy! They do, however, lower their stack energies enough to take the total energy to -0.2, and cause no misfolds.

Placing one GC or CG pair in either closing pair position immediately solves the puzzle. The GC in row 5 of the figure lowers total energy to -1.2, the same as the CG in row 8. The CG in row 6 and the GC in row 7 lower total energy to -1.3.

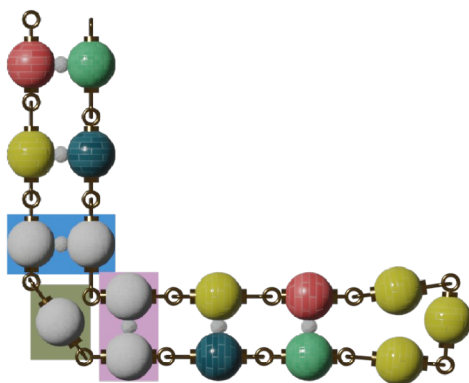
In the `[2.1.2 The double-strand loop, or “regular” loop](#)` section of the “Advanced puzzle solving guide”, player drake178 wrote about internal loops in detail.

Solve a bulge

The size of a bulge affects its energy.

The first unpaired pieces in the bulge, where we would expect to find a terminal mismatch, actually have no effect on bulge energy. In fact, none of the unpaired pieces in a bulge affect bulge energy.

The energy of the bulge is like an offset version of the stacking energy between the closing pairs. So, changes we make to the bulge's closing pairs will move up and down in energy just as with the stacking energies between adjacent pairs in a stack.



Row	Pieces	Sequence	Bulge energy !
1	GC, AU, UA, A, AU, UA, CG, A, A, A	GAUAAUCAAAAGUAUC	+2.5
2	GC, AU, UA, U, AU, UA, CG, A, A, A	GAUUAUCAAAAGUAUC	+2.5
3	GC, AU, UA, G, AU, UA, CG, A, A, A	GAUGAUCAAAAGUAUC	+2.5
4	GC, AU, UA, C, AU, UA, CG, A, A, A	GAUCAUCAAAAGUAUC	+2.5
5	GC, AU, UA, A, AU, UA, CG, A, A, A	GAUAAUCAAAAGUAUC	+2.5
6	GC, AU, AU, A, UA, UA, CG, A, A, A	GAAAUUCAAAAGAAUUC	+2.7
7	GC, AU, UG, A, GU, UA, CG, A, A, A	GAUAGUCAAAAGAUUC	+4.1
8	GC, AU, GU, A, UG, UA, CG, A, A, A	GAGAUUCAAAAGAUUC	+5.1
9	GC, AU, GC, A, UG, UA, CG, A, A, A	GAGAUUCAAAAGAGCUC	+1.3 !
10	GC, AU, GU, A, CG, UA, CG, A, A, A	GAGACUCAAAAGAUUC	+1.3
11	GC, AU, GU, U, CG, UA, CG, A, A, A	GAGUCUCAAAAGAUUC (((·(((·))) 1 1234567890123456	+1.3 !

Figure 37. Solving a 1-0 bulge.

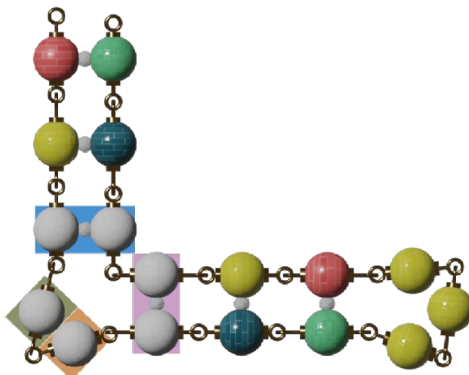
The structure in the figure above has a 3- stack, 1-0 bulge, 3- stack, and 3- hairpin loop. The stacks and hairpin loop sum to a -1.3 energy, but the bulge energy is +2.5 (see row 1), giving a total energy of +1.2. We need the bulge energy to reach +1.3 or lower.

Changing the unpaired piece in the bulge (sequence position 4) has no effect whatsoever on the bulge energy (rows 2 through 4), even though this position is where we would expect terminal mismatch behavior if this were an internal loop.

Let's try changing the closing pairs. This does change the bulge energy, as we can see in rows 5 through 10 in the figure. The row 9 sequence achieves a +1.3 bulge energy and solves the puzzle. Row 10, though, causes a misfold when the A in the bulge decides to pair with the U (at sequence position 14) in the GU closing pair. Placing a U in the bulge (at position 4) blocks the pairing and solves the puzzle (row 11) without affecting bulge energy.

Also, we notice a pattern here that we didn't see for internal loop energies. Here, when we change the bulge's closing pairs, the bulge energy changes by the same amount as if there was no bulge and we were looking at the stacking energy between just these closing pairs. (See the table in "Swap paired pieces".) The UA,AU pairs yield a +2.5 bulge energy (row 5 here) and a -1.3 stacking energy (row 25 there). The AU,UA pairs yield a +2.7 bulge energy (row 6 here) and a -1.1 stacking energy (row 26 there). That's a change of +0.2 between the two rows both here and there. Similarly, changing from AU,UA (rows 6 and 26) to UG,GU (rows 7 and 35) is +1.4. From UG,GU to GU,UG (rows 8 and 36) is +1.0; from GU,UG to GC,UG (rows 9 and 5) is -3.8; and from GC,UG to GU,CG (rows 10 and 4) is 0.0.

This behavior implies that the stacking energies table will help in solving 1-0 bulges and 0-1 bulges.



Row	Pieces	Sequence	Bulge energy !
1	GC, AU, UA, A, A, AU, UA, CG, A, A, A	GAUAAAUCAAAAGAUUC	+3.8
2	GC, AU, UA, U, A, AU, UA, CG, A, A, A	GAUUAUCAAAAGAUUC	+3.8
3	GC, AU, UA, G, A, AU, UA, CG, A, A, A	GAUGAAUCAAAAGAUUC	+3.8
4	GC, AU, UA, C, A, AU, UA, CG, A, A, A	GAUCAAUCAAAGAUUC	+3.8
5	GC, AU, UA, A, U, AU, UA, CG, A, A, A	GAUAUAUCAAAAGAUUC	+3.8
6	GC, AU, UA, A, G, AU, UA, CG, A, A, A	GAUAGAUCAAAGAUUC	+3.8
7	GC, AU, UA, A, C, AU, UA, CG, A, A, A	GAUACAUCAAAGAUUC	+3.8
8	GC, AU, UA, A, A, AU, UA, CG, A, A, A	GAUAAAUCAAAAGAUUC	+3.8
9	GC, AU, AU, A, A, UA, UA, CG, A, A, A	GAAAAUCAAAGAAUUC	+3.8
10	GC, AU, UG, A, A, GU, UA, CG, A, A, A	GAUAAGUCAAAAGAUUC	+3.8
11	GC, AU, GU, A, A, UG, UA, CG, A, A, A	GAGAAUCAAAGAGUUC	+3.8
12	GC, AU, GC, A, A, CG, UA, CG, A, A, A	GAGAACUCAAAAGAGCUC	+2.8 !
13	GC, AU, GC, A, A, GC, UA, CG, A, A, A	GAGAAGUCAAAAGACCUC	+2.8 !
14	GC, AU, CG, A, A, GC, UA, CG, A, A, A	GACAAGUCAAAAGACGUC	+2.8
15	GC, AU, CG, A, A, CG, UA, CG, A, A, A	GACAACUCAAAAGAGGUC	+2.8

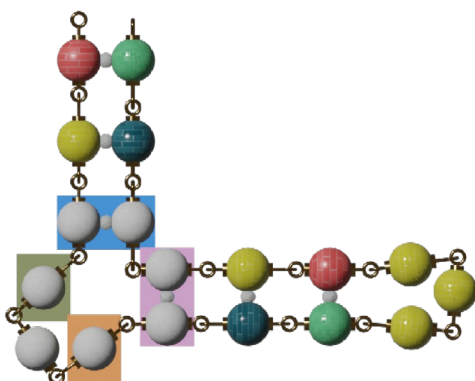
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Figure 38. Solving a 2-0 bulge.

The structure in the figure above features a 2-0 bulge, but otherwise is the same as the 1-0 bulge before.

Like the 1-0 bulge, changing unpaired pieces in this 2-0 bulge (rows 2 through 7) has no effect on the bulge energy.

Unlike the 1-0 bulge, changing closing pairs sometimes does not change the bulge energy at all, as we can see in rows 8 through 11. A GC,CG set (row 12) does change the bulge energy and solves the puzzle, as does a GC,GC set (row 13). A CG,GC closing pair set (row 14), though, does not actually close the GC,AU,CG stack (sequence positions 1-3 and 15-17). And, a CG,CG set (row 15) causes a misfold when the C in position 6 pairs with the G in position 12.



Row	Pieces	Sequence	Bulge energy !
1	GC, AU, UA, A, A, A, AU, UA, CG, A, A, A	GAUAAAUCAAAAGAUUC	+4.2
2	GC, AU, UA, A, U, A, AU, UA, CG, A, A, A	GAUAUAAUCAAAAGAUUC	+4.2
3	GC, AU, UA, A, G, A, AU, UA, CG, A, A, A	GAUAGAAUCAAAAGAUUC	+4.2
4	GC, AU, UA, A, C, A, AU, UA, CG, A, A, A	GAUACAAUCAAAAGAUUC	+4.2
5	GC, AU, UA, A, A, A, AU, UA, CG, A, A, A	GAUAAAUCAAAAGAUUC	+4.2
6	GC, AU, AU, A, A, A, UA, UA, CG, A, A, A	GAAAAAUUCAAAAGAAUUC	+4.2
7	GC, AU, UG, A, A, A, GU, UA, CG, A, A, A	GAUAAAGUCAAAAGAUUC	+4.2
8	GC, AU, GU, A, A, A, UG, UA, CG, A, A, A	GAGAAAUUCAAAAGAGUUC	+4.2
9	GC, AU, GC, A, A, A, CG, UA, CG, A, A, A	GAGAAACUCAAAAGAGCUC	+3.2
10	GC, AU, GC, A, A, A, GC, UA, CG, A, A, A	GAGAAAGUCAAAAGACCUC	+3.2
11	GC, AU, CG, A, A, A, GC, UA, CG, A, A, A	GACAAAAGUCAAAAGACGUC	+3.2
12	GC, AU, CG, A, A, A, CG, UA, CG, A, A, A	GACAAACUCAAAAGAGGUC	+3.2

(((...(((...))))))
1
123456789012345678

Figure 39. Solving a 3-0 bulge.

The structure in the figure above features a 3-0 bulge, but otherwise is the same as the 2-0 bulge before.

Since the unpaired pieces just inside the bulge (sequence positions 4 and 6) had no effect for the previous 2-0 bulge, we do not expect them to change the bulge energy here in this 3-0 bulge, and this is true. Further, changing the middle bulge piece (position 5) also has no effect on bulge energy, as rows 2 through 4 in the figure show.

For this 3-0 bulge, we try the same closing pair combinations that we tried for the 2-0 bulge. Here as there, combinations of AU, UA, UG, and GU do not change bulge energy at all. All of the GC and CG combinations change the bulge energy to +3.2. The GC,GC closing pair set (row 10) does solve the puzzle. The GC,CG set (row 9) misfolds radically when G,A,G in positions 1-3 pair with C,U,C in positions 7-9. The CG,GC set (row 11) and the CG,CG set (row 12) simply fail to actually close the would-be stack in positions 1-3.

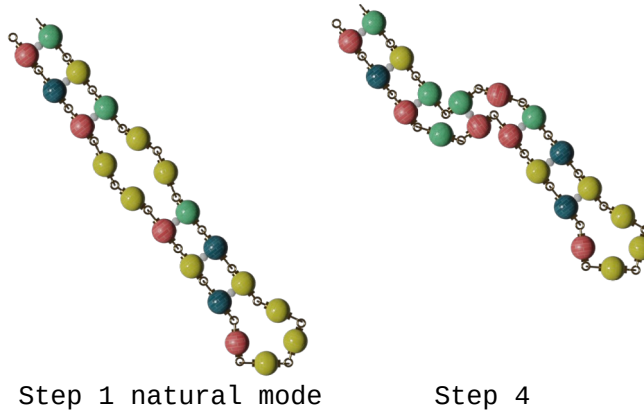
Solve a zigzag

Solving a zigzag presents the challenge that each inner closing pair must close two bulges at the same time.

To solve zigzag puzzles, we try two tactics at the same time. In target viewing mode, we want to lower the energy sum of bulges making up the zigzag. In natural viewing mode, we want to raise the energy sum of the internal loops that are misfolds of the zigzag. Zigzags share characteristics with both internal loops and bulges, so “Boost a loop” and “Swap paired pieces” will help.

Let's work on a 1-0,0-1 zigzag.

Step	Sequence	!
1	GUGAAGAUGAAAAUCAACAC	
2	GUGAGGAUGAAAAUCCAC	
3	GUGAGGAUGAAAAUCGCCAC	
4	GUGCGGAUGAAAAUCGCCAC	!
	(((.(((.....))).)))	← Target structure.
	1 2	
	12345678901234567890	



Step	Natural mode pieces
1	GC, UA, GC, A, A, GC, AU, UA, G, A, A, A, {UA}, {AU}, {GC}, A, A
2	GC, UA, GC, A, G, GC, AU, UA, G, A, A, A, {UA}, {AU}, {GC}, A, C
3	GC, UA, GC, A, G, GC, AU, UA, G, A, A, A, {UA}, {AU}, {GC}, G, C
4	GC, UA, GC, C, GC, GC, AU, UA, G, A, A, A, {UA}, {AU}, {GC}, G

Step	Target mode pieces
1	GC, UA, GC, A, AA, GC, AU, UA, G, A, A, A, {UA}, {AU}, {GC}, A
2	GC, UA, GC, A, GC , GC, AU, UA, G, A, A, A, {UA}, {AU}, {GC}, A
3	GC, UA, GC, A, GC, GC, AU, UA, G, A, A, A, {UA}, {AU}, {GC}, G
4	GC, UA, GC, C , GC, GC, AU, UA, G, A, A, A, {UA}, {AU}, {GC}, G

Figure 41. Solving a 1-0,0-1 zigzag.

In the figure above, the target structure we desire has a 3- stack, a 1-0,0-1 zigzag, another 3- stack, and a 4- hairpin loop. We start with an intermediate sequence that shows a 3- stack, 2-2 internal loop, 3- stack, and 4- hairpin loop in natural viewing mode (step 1 in the figure). The stacks and hairpin loop are all stable when flipping between target mode and natural mode, and the only change we see is from zigzag to internal loop and back.

The target mode wants a pair of some kind at sequence positions 5 and 17, so let's start there. There is a GC pair at 3,18 and another GC pair at 6,15. The stacking energy table in "Swap paired pieces" shows a GC,CG set at -3.4 and a CG,GC set at -2.4 (a 1.0 difference). If we put a CG pair at 5,17 in target mode we see +0.4 and +1.4 bulge energies (also a 1.0 difference) for a sum of +1.8. The stacking energy table also shows a GC,GC set at -3.3. If we put a GC pair at 5,17 in target mode, we see +0.5 and +0.5 bulge energies (a 0.0 difference — but that makes sense if we consider they are the same set), for a sum of +1.0. So, we place a GC pair (step 2) because it gives a lower bulge energy sum (+1.0) than placing a CG pair (+1.8).

We flip now to normal mode, and see that the 2-2 loop has a -1.0 energy. We'll try changing the loop energy by placing pieces in the terminal mismatch positions. Some of the pieces will cause a misfold, but we find that a U at position 4 gives a +0.2 loop energy, or that a G at position 16 gives a +0.8 loop energy. We'll choose the G at 16 for the higher loop energy (step 3).

Still in normal viewing mode, we try U, then G, then C at position 4. The U lowers loop energy from +0.8 to +0.5. The G causes a misfold. The C raises the loop energy enough to solve the puzzle (step 4)!

Here are some examples of zigzag puzzle solutions.

Row	Pieces	Sequence	Total energy !
1	AU, UA, GC, C, GC, CG, UA, GC, A, A, A, A, {GC}, {UA}, {CG}, A	AUGCUCUGAAAACAGACCAU	-1.5 !
2	AU, UA, GC, A, CG, CG, UA, GC, A, A, A, A, {GC}, {UA}, {CG}, C	AUGACCUGAAAACAGCGCAU	-1.5 !
3	GC, UA, GC, C, GC, GC, AU, UA, G, A, A, A, {UA}, {AU}, {GC}, G	GUGCUGAUGAAAUCGCCAC (((.((((.....))).))) 1 2 12345678901234567890	-4.1 !

Figure 42. Some solutions for a 1-0,0-1 zigzag.¹⁴

Row	Pieces	Sequence	Total energy !
1	CG, AU, UA, UA, C, GC, GC, UA, CG, G, A, A, A, {CG}, {UA}, {GC}, G	CAUUCGGUCGAAAGACGCAAUG	-6.1 !
2	CG, AU, UA, CG, G, CG, AU, UA, CG, G, A, A, A, {CG}, {UA}, {AU}, C	CAUCGCAUCGAAAGAUCGGAUG	-6.5 !
3	CG, AU, UA, CG, G, CG, CG, UA, AU, A, A, A, A, {AU}, {UA}, {CG}, C	CAUCGCCUAAAAAUAGCGGAUG (((.((((.....))).))) 1 2 1234567890123456789012	-2.7 !

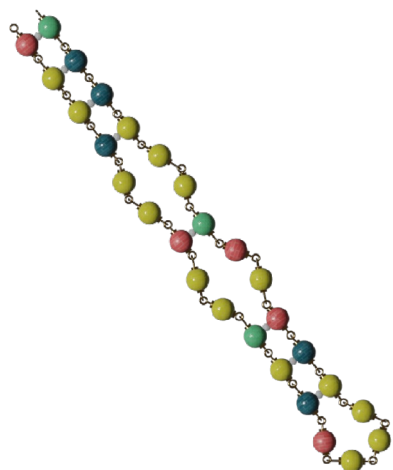
Figure 43. More solutions for a 1-0,0-1 zigzag.¹⁵

14 Adapted from “Zig”, “Zig 2”, and “Zig 5” in “Zigzags” section of “Tips and trick guide” by Eli Fisker. (Also see the “Mat - Small zig” Eterna puzzle.)

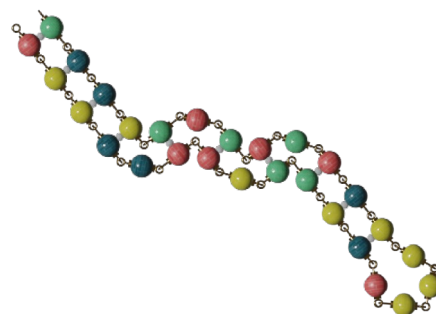
15 Adapted from “Zig3”, “Zig 4”, and “Zig 6” in “Zigzags” section of “Tips and trick guide” by Eli Fisker. (Also see the “Mat - Small zig” Eterna puzzle.)

Let's work on a longer zigzag.

Step	Sequence	!
1	GAAUAAGAACAUGAAAAUGAGCAAUUC	
2	GAAUAAGACCAUGAAAAUGAGCAAUUC	
3	GAAUAAGACCAUGAAAAUGC G CAAUUC	
4	GAAUA G GACCAUGAAAAUGCGCA C AUUC	
5	GAAUAGGACCAUGAAAAUGCGC G CAUUC	
6	GAAU U GGACCAUGAAAAUGCGCGCAUUC	!
	(((.(.(((.....)).)).))))	← Target structure.
	<div style="display: flex; justify-content: space-around; width: 100%;"> 1 2 </div>	
	1234567890123456789012345678	



Step 1 natural mode



Step 6

Step	Natural mode pieces
1	GC, AU, AU, UA, A, A, GC, A, A, CG, AU, UA, G, A, A, A, {UA}, {AU}, {CG}, A, G, {GC}, A, A
2	GC, AU, AU, UA, A, A, GC, A, C , CG, AU, UA, G, A, A, A, {UA}, {AU}, {CG}, A, G, {GC}, A, A
3	GC, AU, AU, UA, A, A, GC, A, CG , CG, AU, UA, G, A, A, A, {UA}, {AU}, {CG}, C , {CG}, {GC}, A, A
4	GC, AU, AU, UA, A, GC , G, A, CG, CG, AU, UA, G, A, A, A, {UA}, {AU}, {CG}, C, {CG}, {GC}, A, GC
5	GC, AU, AU, UA, A, G, GC, A, CG, CG, AU, UA, G, A, A, A, {UA}, {AU}, {CG}, C, {CG}, {GC}, G , C
6	GC, AU, AU, UA, U , GC, GC, A, CG, CG, AU, UA, G, A, A, A, {UA}, {AU}, {CG}, C, {CG}, {GC}, G

Step	Target mode pieces
1	GC, AU, AU, UA, A, AA , GC, A, AG , CG, AU, UA, G, A, A, A, {UA}, {AU}, {CG}, A, AG , {GC}, A
2	GC, AU, AU, UA, A, AA , GC, A, CG , CG, AU, UA, G, A, A, A, {UA}, {AU}, {CG}, A, CG , {GC}, A
3	GC, AU, AU, UA, A, AA , GC, A, CG , CG, AU, UA, G, A, A, A, {UA}, {AU}, {CG}, C , {CG}, {GC}, A
4	GC, AU, AU, UA, A, GC , GC, A, CG, CG, AU, UA, G, A, A, A, {UA}, {AU}, {CG}, C, {CG}, {GC}, A
5	GC, AU, AU, UA, A, GC, GC, A, CG, CG, AU, UA, G, A, A, A, {UA}, {AU}, {CG}, C, {CG}, {GC}, G
6	GC, AU, AU, UA, U , GC, GC, A, CG, CG, AU, UA, G, A, A, A, {UA}, {AU}, {CG}, C, {CG}, {GC}, G

Note: AA and AG do not pair in game. We do this here to illustrate in target mode.

Figure 44. Solving a 1-0,0-1,1-0,0-1 zigzag.¹⁶

¹⁶ Adapted from “Zigzag, really big” in “Zigzags” section of “Tips and trick guide” by Eli Fisker. (See also the “Big Zig Zag” Eterna puzzle.)

In the figure above, our desired target structure has a 4- stack, a 1-0,0-1,1-0,0-1 zigzag, a 3- stack, and a 4- hairpin loop. We start with an intermediate sequence that in natural mode shows us a 4- stack, 2-2 loop, 1- stack, 2-2 loop, 3- stack, and a 4- hairpin loop (step 1 in the figure). The stacks and the hairpin loop are all stable between target mode and natural mode; and we can flip between the internal loops (natural mode) and the zigzag (target mode).

In target mode, we place a C at sequence position 9 (step 2) to make GC,CG and CG,CG sets in the third and fourth bulges in target mode. (The GC,CG set has the strongest stacking energy, and the CG,CG set is only 0.1 more positive.) In natural mode, however, we still have a 2-2 loop with -0.2 energy.

Still in natural mode, we place a C at position 20 (step 3) to raise the loop toward more positive energy, and we see the natural mode loop collapsing into the two bulges we wanted there.

In natural mode, we see a 2-2 internal loop near position 5 with a +2.0 loop energy, and the bulge near position 8 with +0.4 energy. Together, that loop and bulge have +2.4 energy.

Flipping to target mode, we place G at 6 and C at 24 (step 4) to make UA,GC and GC,GC sets in the first and second bulges in target mode. In natural mode, this makes a bulge near position 5 with +1.7 energy and an internal loop near position 7 with -0.8 energy, summing to +0.9.

In natural mode, we place a G at 23 (step 5), which changes energies enough to allow the previous internal loop and bulge to form again, but now with $+1.8+0.4 = +2.2$ energy (instead of the +2.4 sum it had in step 3).

Still in natural mode, we place a U at sequence position 5 (step 6). This raises the loop energy from +1.8 enough to allow the GC pair at 6,24 to form, and incidentally solves the puzzle.

Solve a hairpin loop

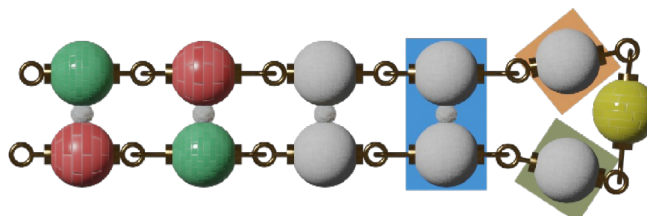
Solving a hairpin loop is much like solving an internal loop, but worrying about only one closing pair instead of two. As with an internal loop, we focus on the closing pair and potential terminal mismatches in a hairpin loop. We aim to lower either the closing pair's stacking energy or the hairpin loop's energy, or both, to solve the puzzle.

The game does allow 1 piece or 2 piece hairpin structures, but makes their energy so high that they become unworkable for solving puzzles.

We begin, then, with a 3 piece hairpin loop (“3- hairpin”), also called a triloop.

((((. . .)))

Figure 45. Closing pair and terminal mismatch positions for a 3- hairpin loop.



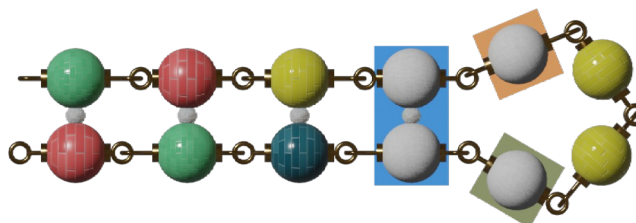
Row	Pieces	Sequence	Stack energy	Loop energy	Total energy	!
1	GC, CG, UA, AU, A, A, A	GCUAAAAUAGC	-6.8	+6.2	-0.6	!
2	GC, CG, AU, UA, A, A, A	GCAUAAAAUAGC	-6.6	+6.2	-0.4	!
3	GC, CG, UA, UG, A, A, A	GCUUAAAAGAGC	-6.8	+6.2	-0.6	!
4	GC, CG, UA, GU, A, A, A	GCUGAAAUAGC	-6.5	+6.2	-0.3	!
5	GC, CG, UA, GC, A, A, A	GUCGAAACAGC	-7.6	+5.7	-1.9	!
6	GC, CG, UA, CG, A, A, A	GCUCAAAAGAGC	-7.9	+5.7	-2.2	!
7	GC, CG, UA, CG, A, A, U	GCUCAAUAGAGC	-7.9	+5.7	-2.2	!
8	GC, CG, UA, CG, A, A, G	GCUCAAGGAGC	-7.9	+5.7	-2.2	!
9	GC, CG, UA, CG, A, A, C	GCUCAACGAGC	-7.9	+5.7	-2.2	!
10	GC, CG, UA, CG, U, A, A	GCUCUAAGAGC	-7.9	+5.7	-2.2	!
11	GC, CG, UA, CG, U, A, U	GCUCUAUAGAGC	-7.9	+5.7	-2.2	!
12	GC, CG, UA, CG, U, A, G	GCUCUAGGAGC	-7.9	+5.7	-2.2	!
13	GC, CG, UA, CG, U, A, C	GCUCUACGAGC	-7.9	+5.7	-2.2	!
14	GC, CG, UA, CG, G, A, A	GCUCGAAGAGC	-7.9	+5.7	-2.2	!
15	GC, CG, UA, CG, G, A, U	GCUCGAUAGAGC	-7.9	+5.7	-2.2	!
16	GC, CG, UA, CG, G, A, G	GCUCGAGGAGC	-7.9	+5.7	-2.2	!
17	GC, CG, UA, CG, G, A, C	GCUCGACGAGC	-7.9	+5.7	-2.2	!
18	GC, CG, UA, CG, C, A, A	GCUCCAAGAGC	-7.9	+5.7	-2.2	!
19	GC, CG, UA, CG, C, A, U	GCUCCAUGAGC	-7.9	+5.7	-2.2	!
20	GC, CG, UA, CG, C, A, G	GCUCCAGGAGC	-7.9	+5.7	-2.2	!
21	GC, CG, UA, CG, C, A, C	GCUCCACGAGC	-7.9	+5.7	-2.2	!

(((. . .)))
1
12345678901

Figure 46. Some solutions for a 3- hairpin loop (a triloop) next to a 4- stack.

In the figure above, we reach two conclusions for a 3- hairpin loop next to a 4- stack. First, all six pairs can serve as the closing pair. Second, none of the pieces in terminal mismatch positions has an effect on loop energy. (This is the origin of the saying that we can't boost a triloop.)

We turn next to a 4 piece hairpin loop (“4- hairpin”), also called a tetraloop.



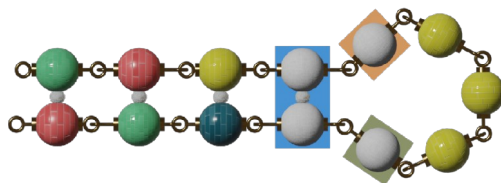
Row	Pieces	Sequence	Stack energy	Loop energy	Total energy	!
1	GC, CG, UA, AU, A, A, A, A	GCUAAAAUAGC	-6.8	+5.3	-1.5	!
2	GC, CG, UA, UA, A, A, A, A	GCUUAAAAAGC	-6.4	+5.1	-1.3	!
3	GC, CG, UA, UG, A, A, A, A	GCUUAAAAAGC	-6.8	+5.1	-1.7	!
4	GC, CG, UA, GU, A, A, A, A	GCUGAAAAUAGC	-6.5	+5.8	-0.7	!
5	GC, CG, UA, GC, A, A, A, A	GCUGAAAAAGC	-7.6	+4.5	-3.1	!
6	GC, CG, UA, CG, A, A, A, A	GCUCAAAAAGC	-7.9	+4.1	-3.8	!
7	GC, CG, UA, CG, A, A, A, U	GCUCAAAUGAGC	-7.9	+3.8	-4.1	!
8	GC, CG, UA, CG, A, A, A, G	GCUCAAAGGAGC	-7.9	+4.2	-3.7	!
9	GC, CG, UA, CG, A, A, A, C	GCUCAAACGAGC	-7.9	+4.1	-3.8	!
10	GC, CG, UA, CG, U, A, A, A	GCUCUAAAGAGC	-7.9	+3.9	-4.0	!
11	GC, CG, UA, CG, U, A, A, U	GCUCUAAUGAGC	-7.9	+3.6	-4.3	!
12	GC, CG, UA, CG, U, A, A, G	GCUCUAAAGGAGC	-7.9	+3.8	-4.1	!
13	GC, CG, UA, CG, U, A, A, C	GCUCUAACGAGC	-7.9	+2.2	-5.7	!
14	GC, CG, UA, CG, G, A, A, A	GCUCGAAAGAGC	-7.9	+0.4	-7.5	!
15	GC, CG, UA, CG, G, A, A, U	GCUCGAAUGAGC	-7.9	+4.5	-3.4	!
16	GC, CG, UA, CG, G, A, A, G	GCUCGAAGGAGC	-7.9	+1.5	-6.4	!
17	GC, CG, UA, CG, G, A, A, C	GCUCGAACGAGC	-7.9	+3.6	-4.3	!
18	GC, CG, UA, CG, C, A, A, A	GCUCCAAAGAGC	-7.9	+4.6	-3.3	!
19	GC, CG, UA, CG, C, A, A, U	GCUCCAAUGAGC	-7.9	+4.8	-3.1	!
20	GC, CG, UA, CG, C, A, A, G	GCUCCAAGGAGC	-7.9	+2.7	-5.2	!
21	GC, CG, UA, CG, C, A, A, C	GCUCCAACGAGC	-7.9	+4.7	-3.2	!

((((.....))))
1
123456789012

Figure 47. Some solutions for a 4- hairpin loop (a tetraloop) next to a 4- stack.

All six pairs can serve as the closing pair in the 4- stack 4- hairpin structure. The pieces in terminal mismatch positions now have an effect on loop energy.

Next is a 5 piece hairpin loop (“5- hairpin”), also called a pentaloop.



Row	Pieces	Sequence	Stack energy	Loop energy	Total energy	!
1	GC, CG, UA, AU, A, A, A, A, A	GCUAAAAAUAGC	-6.8	+5.3	-1.5	!
2	GC, CG, UA, UA, A, A, A, A, A	GCUUAAAAAAGC	-6.4	+5.1	-1.3	!
3	GC, CG, UA, UG, A, A, A, A, A	GCUUAAAAAGAGC	-6.8	+5.1	-1.7	!
4	GC, CG, UA, GU, A, A, A, A, A	GCUGAAAAAUAGC	-6.5	+5.8	-0.7	!
5	GC, CG, UA, GC, A, A, A, A, A	GCUGAAAAACAGC	-7.6	+4.5	-3.1	!
6	GC, CG, UA, CG, A, A, A, A, A	GCUCAAAAAGAGC	-7.9	+4.1	-3.8	!
7	GC, CG, UA, CG, A, A, A, A, U	GCUCAAAAUGAGC	-7.9	+3.8	-4.1	!
8	GC, CG, UA, CG, A, A, A, A, G	GCUCAAAAGGAGC	-7.9	+4.2	-3.7	!
9	GC, CG, UA, CG, A, A, A, A, C	GCUCAAAACGAGC	-7.9	+4.1	-3.8	!
10	GC, CG, UA, CG, U, A, A, A, A	GCUCUAAAAGAGC	-7.9	+3.9	-4.0	!
11	GC, CG, UA, CG, U, A, A, A, U	GCUCUAAAUGAGC	-7.9	+3.6	-4.3	!
12	GC, CG, UA, CG, U, A, A, A, G	GCUCUAAAAGGAGC	-7.9	+3.8	-4.1	!
13	GC, CG, UA, CG, U, A, A, A, C	GCUCUAAACGAGC	-7.9	+4.2	-3.7	!
14	GC, CG, UA, CG, G, A, A, A, A	GCUCGAAAAGAGC	-7.9	+3.4	-4.5	!
15	GC, CG, UA, CG, G, A, A, A, U	GCUCGAAAUGAGC	-7.9	+4.5	-3.4	!
16	GC, CG, UA, CG, G, A, A, A, G	GCUCGAAAAGGAGC	-7.9	+4.0	-3.9	!
17	GC, CG, UA, CG, G, A, A, A, C	GCUCGAAACGAGC	-7.9	+3.6	-4.3	X
18	GC, CG, UA, CG, C, A, A, A, A	GCUCCAAAGAGC	-7.9	+4.6	-3.3	!
19	GC, CG, UA, CG, C, A, A, A, U	GCUCCAAUGAGC	-7.9	+4.8	-3.1	!
20	GC, CG, UA, CG, C, A, A, A, G	GCUCCAAAGGAGC	-7.9	+2.7	-5.2	X
21	GC, CG, UA, CG, C, A, A, A, C	GCUCCAAACGAGC	-7.9	+4.7	-3.2	!

(((.....)))
1
1234567890123

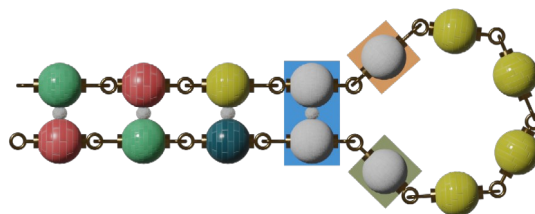
Figure 48. Some solutions for a 5- hairpin loop (a pentaloop) next to a 4- stack.

Again, all six pairs can close the loop in a 4- stack 5- hairpin structure. And again, unpaired pieces in terminal mismatch positions affect loop energy. Note that our sequences for rows 17 and 20 do not achieve success. The G and C in their terminal mismatch positions pair together, misfolding the pentaloop into a triloop. Why? For row 17, the target mode shows a pentaloop with +3.6 energy. Its natural mode, however, shows a CG,GC stacking energy¹⁷ of -2.4 and a triloop energy of +5.7, which sum to +3.3. The +3.3 sum is lower than the +3.6 sum, so the sequence misfolds into the natural mode structure. For row 20, the target mode’s pentaloop has +2.7 energy. Its natural mode’s CG,CG stacking energy¹⁸ (-3.3) and triloop energy (+5.7) sum to +2.4, so we have another misfold.

17 See row 7 in the “Swap paired pieces” section’s stacking energies table.

18 See row 2 in the “Swap paired pieces” section’s stacking energies table.

Finally, we explore a 6 piece hairpin loop (“6- hairpin”), also called a hexaloop.



Row	Pieces	Sequence	Stack energy	Loop energy	Total energy	!
1	GC, CG, UA, AU, A, A, A, A, A, A	GCUAAAAAAAAUAGC	-6.8	+5.1	-1.7	!
2	GC, CG, UA, UA, A, A, A, A, A, A	GCUUAAAAAAAAAGC	-6.4	+4.9	-1.5	!
3	GC, CG, UA, UG, A, A, A, A, A, A	GCUUAAAAAAAAAGC	-6.8	+4.9	-1.9	!
4	GC, CG, UA, GU, A, A, A, A, A, A	GCUGAAAAAAAAUAGC	-6.5	+5.6	-0.9	!
5	GC, CG, UA, GC, A, A, A, A, A, A	GCUGAAAAAACAGC	-7.6	+4.3	-3.3	!
6	GC, CG, UA, CG, A, A, A, A, A, A	GCUCAAAAAAGAGC	-7.9	+3.9	-4.0	!
7	GC, CG, UA, CG, A, A, A, A, A, U	GCUCAAAAAUGAGC	-7.9	+3.6	-4.3	X
8	GC, CG, UA, CG, A, A, A, A, A, G	GCUCAAAAAGGAGC	-7.9	+4.0	-3.9	!
9	GC, CG, UA, CG, A, A, A, A, A, C	GCUCAAAAACGAGC	-7.9	+3.9	-4.0	!
10	GC, CG, UA, CG, U, A, A, A, A, A	GCUCUAAAAAGAGC	-7.9	+3.7	-4.2	X
11	GC, CG, UA, CG, U, A, A, A, A, U	GCUCUAAAAUGAGC	-7.9	+3.4	-4.5	!
12	GC, CG, UA, CG, U, A, A, A, A, G	GCUCUAAAAAGGAGC	-7.9	+3.6	-4.3	X
13	GC, CG, UA, CG, U, A, A, A, A, C	GCUCUAAAAACGAGC	-7.9	+4.0	-3.9	!
14	GC, CG, UA, CG, G, A, A, A, A, A	GCUCGAAAAAGAGC	-7.9	+3.2	-4.7	!
15	GC, CG, UA, CG, G, A, A, A, A, U	GCUCGAAAAUGAGC	-7.9	+4.3	-3.6	!
16	GC, CG, UA, CG, G, A, A, A, A, G	GCUCGAAAAAGGAGC	-7.9	+3.8	-4.1	!
17	GC, CG, UA, CG, G, A, A, A, A, C	GCUCGAAAAACGAGC	-7.9	+3.4	-4.5	X
18	GC, CG, UA, CG, C, A, A, A, A, A	GCUCCAAAAAGAGC	-7.9	+4.4	-3.5	!
19	GC, CG, UA, CG, C, A, A, A, A, U	GCUCCAAAAUGAGC	-7.9	+4.6	-3.3	!
20	GC, CG, UA, CG, C, A, A, A, A, G	GCUCCAAAAAGGAGC	-7.9	+2.5	-5.4	X
21	GC, CG, UA, CG, C, A, A, A, A, C	GCUCCAAAAACGAGC	-7.9	+4.5	-3.4	!

(((.....)))
1
12345678901234

Figure 49. Some solutions for a 6- hairpin loop (a hexaloop) next to a 4- stack.

All six pairs can close the loop in a 4- stack 6- hairpin structure. Unpaired pieces in terminal mismatch positions continue to affect loop energy.

Several rows now do not achieve success. In rows 7 and 10, the A and U in the mismatch positions pair together. The U and G in row 12's sequence pair together; and the G and C in rows 17 and 20 pair together. All these pairings misfold the structure into a smaller hairpin loop. If we compare these misfolds with the “Swap paired pieces” section's stacking energies table, we see a progression through that table's rows: 2 (CG,CG), 7 (CG,GC), 14 (CG,UG), 15 (CG,UA), and 16 (CG,AU). Only row 20 (CG,GU) in the stacking energies table does not cause a misfold at row 15 in the figure above. We are

seeing a linear pattern: above some threshold, stacking energies of potential pairings are not strong enough to cause a misfold. Looking back at the 5- hairpin loop figure, above, we can find this same pattern.

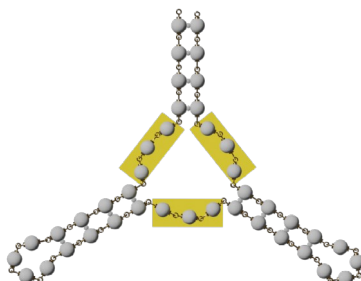
So, we can assume that the stacking energies table is an useful guide to help with hairpin loop terminal mismatches.

Player drake178 studied a 6-piece hairpin loop in detail in “[2.1.1 The Hairpin](#)” of the “Advanced puzzle solving guide”.

Player dimension9 developed a tetraloop reference table in 2011, posted its [final version](#), and discussed it [in a forum thread](#). The reference table “is a list of the most abundant, most frequently occurring tetraloop configurations” found in nature. Tetraloops being commonly found in nature, dimension9 hoped that players could benefit especially in making designs for the Eterna labs.

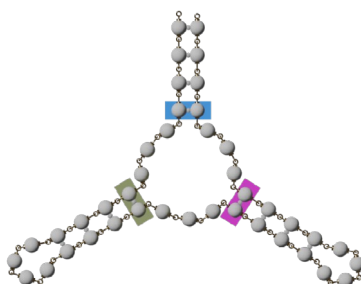
Solve a multibranch loop

Solving a multibranch loop shares techniques with solving an internal loop. We focus on the closing pairs and terminal mismatch positions.



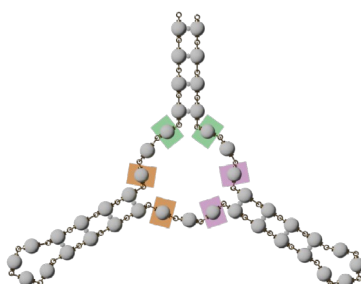
(((...(((.....)))...(((.....)))...))))

Figure 50. Unpaired pieces making up a 3-3-3 multibranch loop.



((([...](((.....)))]...[(((.....)))]...]))))

Figure 51. Closing pair positions for a 3-3-3 multibranch loop.



(((..(((.....)))..(((.....)))..))))

Figure 52. Terminal mismatch positions for a 3-3-3 multibranch loop.

Item	Pieces
0a	GC, CG, UA, AU, GU, UA, CG, GC, A, A, A, A, {GC}, {CG}, {UA}, {GU}, GU, UA, GC, GC, A, A, A, A
1a	GC, CG, UA, AU, A, GU, UA, CG, GC, A, A, A, A, {GC}, {CG}, {UA}, {GU}, A, GU, UA, GC, GC, A, A, A, A, {GC}, {GC}, {UA}, {GU}, A
2a	GC, CG, UA, AU, A, A, GU, UA, CG, GC, A, A, A, A, {GC}, {CG}, {UA}, {GU}, A, A, GU, UA, GC, GC, A, A, A, A, {GC}, {GC}, {UA}, {GU}, A, A
3a	GC, CG, UA, AU, A, A, A, GU, UA, CG, GC, A, A, A, A, {GC}, {CG}, {UA}, {GU}, A, A, A, GU, UA, GC, GC, A, A, A, A, {GC}, {GC}, {UA}, {GU}, A, A, A
4a	GC, CG, UA, AU, A, A, A, A, GU, UA, CG, GC, A, A, A, A, {GC}, {CG}, {UA}, {GU}, A, A, A, A, GU, UA, GC, GC, A, A, A, A, {GC}, {GC}, {UA}, {GU}, A, A, A, A

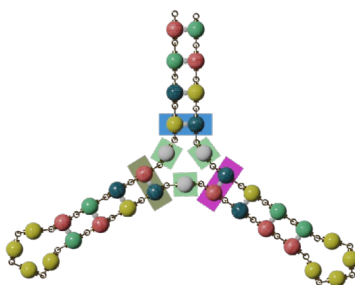
Item	Sequence	Multibranch energy	Total energy	!
0b	GCUAGUCGAAAACGAUGUGGAAAACCAUUAGC (((((((((.....))))))(((.....)))))) 1 2 3 12345678901234567890123456789012	+6.1	-4.7	!
1b	GCUAAGUCGAAAACGAUAGUGGAAAACCAUAUAGC ((((.(((.....))))).(((.....)))))) 1 2 3 12345678901234567890123456789012345	+3.9	-6.9	!
2b	GCUAAAGUCGAAAACGAUAAUGUGGAAAACCAUAAUAGC ((((.(((.....))))..(((.....))))..))) 1 2 3 12345678901234567890123456789012345678	+3.0	-7.8	!
3b	GCUAAAAGUCGAAAACGAUAAAUGUGGAAAACCAUAAAUAGC ((((.(((.....))))...(((.....))))...))) 1 2 3 4 12345678901234567890123456789012345678901	+3.0	-7.8	!
4b	GCUAAAAAGUCGAAAACGAUAAAAGUGGAAAACCAUAAAUAGC ((((.(((.....))))....(((.....))))....))) 1 2 3 4 12345678901234567890123456789012345678901234	+3.0	-7.8	!

Figure 53. Same-stack sequences that solve a 0-0-0 multibranch loop, 1-1-1 multibranch loop, 2-2-2 multibranch loop, 3-3-3 multibranch loop, and 4-4-4 multibranch loop.

The figure above illustrates the closing pair and terminal mismatch positions for multibranch loops of increasing size, and also shows the size change effect on multibranch loop energy. The structure is a set of three 4- stacks (two of the stacks capped by 4- hairpin loops) meeting at a central multibranch loop. The sequences are identical for the stacks and hairpin loops, and change only in the number of the multibranch loop's unpaired pieces.

The 0-0-0 multibranch loop in item 0a and 0b has no unpaired pieces, and therefore no terminal mismatch locations. This is why we can't "Boost a loop" here.

The 1-1-1 multibranch loop item in 1a and 1b has a single unpaired piece between closing pairs. This single piece does act as a terminal mismatch position piece, even though it has no partner to mismatch.



Row	Sequence	Multibranch energy	Total energy	!
1	GCUAAGUCGAAAACGAUAGUGGAAAACCAU <u>A</u> UAGC	+3.9	-6.9	!
2	GCUAUGUCGAAAACGAUAGUGGAAAACCAU <u>U</u> UAGC	+4.1	-6.7	!
3	GCUAAGUCGAAAACGAUUGUGGAAAACCAU <u>A</u> UAGC	+4.4	-6.4	X
4	GCUAAGUCGAAAACGAUAGUGGAAAACCAU <u>U</u> UAGC	+4.4	-6.4	!
5	GCUAUGUCGAAAACGAUAGUGGAAAACCAU <u>U</u> UAGC	+4.6	-6.2	!
6	GCUAGGUCGAAAACGAUAGUGGAAAACCAU <u>A</u> UAGC	+3.9	-6.9	!
7	GCUAAGUCGAAAACGAU <u>G</u> UGGAAAACCAU <u>A</u> UAGC	+3.9	-6.9	X
8	GCUAAGUCGAAAACGAUAGUGGAAAACCAU <u>G</u> UAGC	+3.9	-6.9	!
9	GCUAGGUCGAAAACGAUAGUGGAAAACCAU <u>G</u> UAGC	+3.9	-6.9	!
10	GCUACGUCGAAAACGAUAGUGGAAAACCAU <u>A</u> UAGC	+4.2	-6.6	!
11	GCUAAGUCGAAAACGAU <u>C</u> GUGGAAAACCAU <u>A</u> UAGC	+4.3	-6.5	!
12	GCUAAGUCGAAAACGAUAGUGGAAAACCAU <u>C</u> UAGC	+4.5	-6.3	!
13	GCUACGUCGAAAACGAU <u>C</u> GUGGAAAACCAU <u>A</u> UAGC	+4.6	-6.2	X
14	GCUACGUCGAAAACGAUAGUGGAAAACCAU <u>C</u> UAGC	+4.8	-6.0	!
15	GCUAAGUCGAAAACGAU <u>C</u> GUGGAAAACCAU <u>C</u> UAGC	+4.9	-5.5	!
16	GCUACGUCGAAAACGAU <u>C</u> GUGGAAAACCAU <u>C</u> UAGC	+5.2	-5.6	X

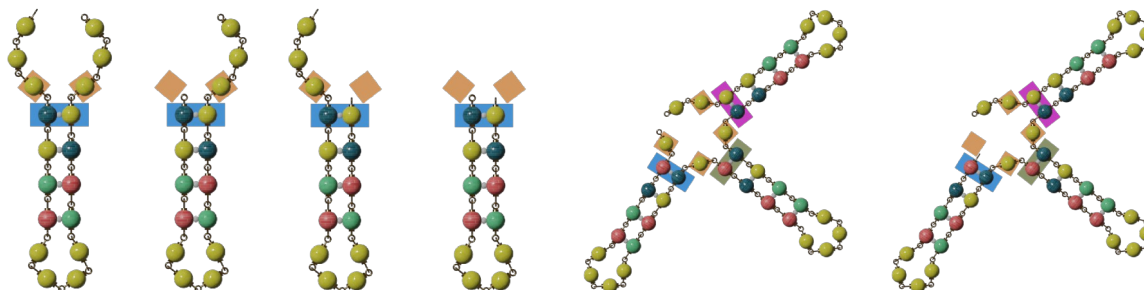
(((.(((....))))).(((....))))))
 1 2 3
 12345678901234567890123456789012345

Figure 54. Changing terminal mismatch position pieces in a 1-1-1 multibranch loop.

In the figure above, we change a 1-1-1 multibranch loop's unpaired pieces to see their effect. Each of the changes from A to U (rows 2 through 5) raises the multibranch loop energy. Row 3's misfold happens when the U at position 18 pairs with A at 31. Changes from A to G (rows 6 through 9) do not alter the multibranch loop energy at all. Row 7 misfolds when the G at 18 pairs with C at 28. Changes from A to C (rows 10 through 16) raise the multibranch loop energy. In the row 13 misfold, C at 5 pairs with G at 19 while C at 18 pairs with G at 6. Row 16 does the same misfold as row 13.

Solve an external loop

Like other loops, solving external loops focuses on closing pair and terminal mismatch positions. We use the same techniques to “Boost a loop” and “Swap paired pieces”.



Item	Pieces
1a	A, A, A, UA, AU, CG, GC, A, A, A, A, {GC}, {CG}, {AU}, {UA}, A, A, A
2a	UA, AU, CG, GC, A, A, A, A, {GC}, {CG}, {AU}, {UA}, A, A, A
3a	A, A, A, UA, AU, CG, GC, A, A, A, A
4a	UA, AU, CG, GC, A, A, A, A
5a	A, GU, UA, CG, GC, A, A, A, A, {GC}, {CG}, {UA}, {GU}, A, GU, UA, GC, GC, A, A, A, A, {GC}, {GC}, {UA}, {GU}, A, UA, UA, GC, GC, A, A, A, A, {GC}, {GC}, {UA}, {UA}, A, A
6a	GU, UA, CG, GC, A, A, A, A, {GC}, {CG}, {UA}, {GU}, A, GU, UA, GC, GC, A, A, A, A, {GC}, {GC}, {UA}, {GU}, A, UA, UA, GC, GC, A, A, A, A, {GC}, {GC}, {UA}, {UA}, A, A

Item	Sequence	External loop energy	Total energy	!
1b	AAAUACGAAAACGUAAAA ...((((.....)))... UACGAAAACGUAAAA ((((.....)))... AAAUACGAAAACGUA ...((((.....)))	-0.6	-2.0	!
2b	UACGAAAACGUAAAA ((((.....)))... AAAUACGAAAACGUA ...((((.....)))	-0.3	-1.7	!
3b	AAAUACGAAAACGUA ...((((.....)))	+0.2	-1.2	!
4b	UACGAAAACGUA ((((.....)))	+0.5	-0.9	!
5b	AGUCGAAAACGAUAGUGGAAAACCAUAUUGGAAAACCAAAA .((((.....))).((((.....))).((((.....))). GUCGAAAACGAUAGUGGAAAACCAUAUUGGAAAACCAAAA ((((.....))).((((.....))).((((.....))).	-1.0	-6.8	!
6b	GUCGAAAACGAUAGUGGAAAACCAUAUUGGAAAACCAAAA ((((.....))).((((.....))).((((.....))).	-0.7	-6.5	!
	1 2 3 4 12345678901234567890132456789012345678901			

Figure 55. Examples of solved external loops.

The figure above shows a variety of external loops.

Item 1 has a 3-3 external loop, a 4- stack, and a 4- hairpin loop with a solution sequence. The external loop looks like a set of pincers. Altering the closing pair or terminal mismatch positions changes the external loop energy. None of the other external loop positions (1, 2, 17, 18) affects the energy.

Items 2 and 3 act the same as item 1. They just appear to have a hook instead of pincers. Item 2 has a 0-3 external loop, and item 3 has a 3-0 external loop.

Item 4 has a 4- stack and a 4- hairpin loop. In game, a +0.5 external loop energy does appear, hidden directly between the stack's closing pair at positions 1 and 12. (In contrast, a stacking energy appears in the space between the four pieces involved.) This external loop energy does count in the summing of energies in the structure: the stack has $-1.3 -2.2 -2.4 = -5.9$, and the hairpin loop has +4.5. We need the external loop's +0.5 to make the total energy -0.9. The 0-0 external loop does exist.

Item 5 has three 4- stack 4- hairpin loop branches meeting at a central motif. We would normally call this central motif a multibranch loop, but there's a missing part. This is a 1-1-1-2 external loop. (Simply replace the "multibranch loop" label with "external loop".)

Item 6 is item 5 minus the leading unpaired game piece. We would call the central motif in this item a 0-1-1-2 external loop.

SOLVE PARTITIONS

To solve a partition, solve the motifs within the partition, then focus on the motifs joining that partition to other partitions. (See also the exercise in “Partition the structure”.) If instead we focus on the joining motifs first, we can artificially constrain ourselves while trying to solve the motifs within a partition.

Player drake178¹⁹ said, “In any case where a section does not hold if the stacks surrounding it do, it will NOT HOLD no matter what you do with the rest of the puzzle.” drake178 suggested solving the problem spots first, before moving on to the rest of the puzzle. drake178 considered short stacks to be trouble spots, and said that multibranch loops “with 4 or more stacks - and at least 2 {pieces} that are not between the same two stacks - contain more than one trouble spot.” Bulges can be a problem, and zigzags are always a problem (considering zigzags to be a series of short stacks). Multiple stacks having a similar length can be a problem, because they can misfold against each other. drake178 also described a single partition {but says “structure” and “shenanigan” in the source} as “a series of loops and bulges connected by single or double pairs.”

If there are a lot of partitions, we can approach them like we would a classic cardboard puzzle — work the structure's borders first, then work inward to the puzzle center.

Alternatively, we could identify a central, key, or “grounding” partition, then work outward from there.

Eterna has a tool that can help players who have unlocked it: the [puzzle maker](#). From the Eterna home page, navigate to Play > Puzzle Maker. In the puzzle maker, you can paste or design motifs and secondary structure partitions, and then work to solve them at your leisure.

FULFILL PUZZLE CRITERIA

Sometimes, solving the motifs and partitions doesn't finish the puzzle. There may be additional criteria or constraints, such as limits or thresholds on certain game pieces.

It's usually easier to save this step for last, in your game play approach. Previous steps will have done much of the work already.

19 in “[Advanced puzzle solving guide](#)”

GAME TACTICS AND SCENARIOS

CHANGE THE ENERGY

The game uses energy like a statistic (a stat) or characteristic. There is an energy stat between two pairs of game pieces in a stack. There is an energy stat for each loop. There is an overall energy stat for an entire chain or structure.

Negative energy binds together and stabilizes. Positive energy spreads apart and destabilizes. (Now, what would Oddball say about energy waves?²⁰)

There is energy in every loop and stack in the chain. The game will display stack and loop energies, along with the chain's total energy, if the player has unlocked that capability and adjusted the settings. We discuss stack energy in "Swap paired pieces", so let's discuss total energy and loop energy here.

At the start of a game puzzle showing only As, a chain viewed in target mode has a structure appearing to have paired and unpaired pieces, and displaying zero total energy and zero natural/target delta energy. Flipping to natural mode shows the chain lying in an open arc with no paired pieces and zero total energy. There is no difference between the target and natural total energies, so the delta energy is also zero. But in game, zero is not negative, so the puzzle is not solved.

To solve the puzzle, the target mode structure's total energy must become equal to or less than (more negative than) the natural mode structure's total energy. Or said the other way, the natural mode structure's total energy must become equal to or greater than (more positive than) the target mode structure's total energy. (Also, the misfold diagram must have no red — but that's a different topic.)

Switch to target viewing mode and place a U in a stack position, across from an A. Most likely, the game now shows them as a UA or AU pair, and also displays a positive total energy, a positive delta energy (equal to the total energy for now), and two loop energies. (If the game by chance doesn't display a positive total energy, pick a different position for the U, or switch to another puzzle.) In effect, you made a structure that has an isolated pair closing two loops. Adjust game settings and search around the puzzle diagram for the loop energies. One of the loop energies will be for the structure's external loop, even if your structure ends in a stack and you placed the U in the outside closing pair.²¹

Switch back to natural viewing mode: the loops dissipate, and the total energy again shows as zero. The delta energy keeps its former value, reminding that there is more work to do, yet.

²⁰ *Oddball, a character from the movie, Kelly's Heroes.*

²¹ The external loop energy display sometimes hides directly between the pieces of the closing pair.

Flipping between natural and target viewing modes allows us to examine the energies of each structure in detail.

So, that's how the energy information displays behave at the beginning of a game puzzle. How do we manipulate the energies?

Let's build a chain from scratch, focusing on a simple, expanding loop design.

We can build a hairpin loop with a minimum number of pieces. Technically, we can make a 1- hairpin and a 2- hairpin, but the game sets their loop and total energies to a very high level, indicating that these designs are unworkable.

A 3- hairpin becomes the minimal design, then. A five piece chain (a 3- hairpin and a closing pair) will not fold into this structure: even when trying to use the "strongest" closing pair, the target mode total energy remains positive.

So, we have to make a longer stack, to stitch this 3- hairpin together. A 2- stack lowers the total energy, but not enough. A 3- stack finally takes the total energy negative, closes the loop, and solves the puzzle. (See row 5 in the figure below.)

Row	Pieces	Sequence	Loop energy	Total energy
1	CG, A	CAG (.)	over 9000	over 9000 ²²
2	CG, A, A	CAAG (..)	over 9000	over 9000
3	CG, A, A, A	CAAAG (...)	+5.7	+5.7
4	GC, CG, A, A, A	GCAAAGC (((...)))	+5.7	+2.3
5	CG, GC, CG, A, A, A	CGCAAAGCG ((((...))))	+5.7	-0.1
6	CG, GC, CG, A, A, A, A	CGCAAAAGCG ((((....))))	+4.1	-1.7
7	CG, GC, CG, A, A, A, A, A	CGCAAAAAGCG ((((.....))))	+4.1	-1.7
8	CG, GC, CG, A, A, A, A, A, A	CGCAAAAAAGCG ((((.....))))	+3.9	-1.9
9	CG, GC, CG, A, A, A, A, A, A, A	CGCAAAAAAAGCG ((((.....))))	+4.4	-1.4
10	CG, GC, CG, A, A, A, A, A, A, A, A	CGCAAAAAAAAGCG ((((.....))))	+4.1	-1.7
11	CG, GC, CG, A, A, A, A, A, A, A, A, A	CGCAAAAAAAAAGCG ((((.....))))	+4.9	-0.9
12	CG, GC, CG, A, A, A, A, A, A, A, A, A, A	CGCAAAAAAAAAGCG ((((.....))))	+5.0	-0.8
13	CG, GC, CG, A, A, A, A, A, A, A, A, A, A, A	CGCAAAAAAAAAGCG ((((.....))))	+5.1	-0.7
...		
22	CG, GC, CG, A, A, A, A, A, A, A, A, ..., A	CGCAAAAAAAAAGCG ((((.....))))	+5.75	-0.05
23	CG, GC, CG, A, A, A, A, A, A, A, A, ..., A	CGCAAAAAAAAAGCG ((((.....))))	+5.8	+0.00

Figure 56. Building an expanding hairpin loop.

Now, let's stress the structure to see how it behaves.

We start (at row 5) with a structure having a 3- hairpin loop supported by a 3- stack. The loop energy is +5.7, and the total energy is -0.1, just enough to keep the loop closed. The target and natural mode total energies are identical. The delta energy is 0.

We add pieces to the loop, one by one, to watch the effect. Adding the first piece, to make a 4- hairpin loop, gives the biggest energy drop (-1.6), taking the loop energy to +4.1 and the total energy to -1.7. Adding piece 5 doesn't change the energies, but adding piece 6 bottoms out the energies at +3.9 loop energy and -1.9 total energy. Adding pieces 7 and 8 cause a fluctuation. Adding piece 9 gives the biggest energy jump (+0.8). From that point onward, changes in energy suddenly drop to +0.1 per piece

22 Allusion to *Dragon Ball Z* intended!

and diminish from there. The last and smallest change is +0.05, when adding piece 21 takes the total energy to +0.00 and breaks the loop open once again.

In summary, adding pieces to a minimal hairpin loop initially lowers the energy, then raises the energy, first by a lot, then by a little, and by less and less as we add more pieces. Eventually, the loop bursts open.

What happens if we change the loop pieces?

Row	Loop pieces	A energies		U energies		G energies		C energies	
		change	loop	change	loop	change	loop	change	loop
1	3		+5.7		+5.7		+5.7		+5.7
2	4	-1.6	+4.1	-2.1	+3.6	-1.7	+4.0	-1.0	+4.7
3	5	0.0	+4.1	0.0	+3.6	0.0	+4.0	0.0	+4.7
4	6	-0.2	+3.9	-0.2	+3.4	-0.2	+3.8	-0.2	+4.5
5	7	+0.5	+4.4	+0.5	+3.9	+0.5	+4.3	+0.5	+5.0
6	8	-0.3	+4.1	-0.3	+3.6	-0.3	+4.0	-0.3	+4.7
7	9	+0.8	+4.9	+0.8	+4.4	+0.8	+4.8	+0.8	+5.5
8	10	+0.1	+5.0	+0.1	+4.5	+0.1	+4.9	+0.1	+5.6
9	12					+0.1	+5.8
10	21	+0.05	+5.8				
11	23			+0.05	+5.8		
12	33			+0.03	+5.82				

Figure 57. Energies for all four game pieces in a growing hairpin loop with a CG,GC,CG stack.

Changing the As to all Us, Gs, or Cs affects the size of the hairpin loop that finally bursts open. It also affects the initial energy drop magnitude; but after that, the increments are the same across the various pieces.

Overall, larger loops usually have more positive energy.²³

23 A comment from Brouard's "Puzzle Solving Guide".

Changing individual pieces inside a loop can change the loop energy, when near to the closing pair.

Row	Pieces	Sequence	Internal loop energy
1	GC, A, A, CG, A, A, A, {CG}, A, A	GAACAAAGAAC	+1.5
2	GC, A, A, GC, A, A, A, {GC}, A, A	GAAGAAACAAC	+0.5
3	GC, A, A, GC, A, A, A, {GC}, G, A	GAAGAAACGAC	-0.2
4	GC, G, A, GC, A, A, A, {GC}, G, A	GGAGAAACGAC	-1.7
5	GC, G, U, GC, A, A, A, {GC}, G, U	GGUGAAACGUC	-2.4
6	GC, U, G, GC, A, A, A, {GC}, U, G	GUGGAAACUGC	-4.4
7	GC, U, G, CG, A, A, A, {CG}, U, G	GUGC AAA GUGC (.. (...)..)	-4.9

Figure 58. Example for lowering internal loop energy.²⁴

The closing pair itself also affects loop energy. A GC pair will lower loop energy furthest, compared to other closing pairs.²⁵ Player drake178 said²⁶, “Pairs determine the energy of the loops next to them based on the next {piece} in the loop (starting or ending).”

It may be helpful at times to actually raise the energy in a motif or partition, especially to disrupt an unwanted feature. For example, let's say we have a target mode partition consisting of a multibranch loop, an internal loop, and a hairpin loop, together forming an arm in the overall structure. But in natural mode, the partition misfolds into just a big multibranch loop, and the internal loop and hairpin loop dissolve. In target viewing mode, sum the energies of the multibranch loop, internal loop, and hairpin loop. Then change to natural viewing mode and find the energy of the big multibranch loop. We know that we want to lower the target mode's energy sum to a value below the natural mode energy. If we can no longer adjust the game pieces in the target mode's internal loop and hairpin loop, we are left only with the option to raise the energy of the natural mode's big multibranch loop.²⁷

We can associate an energy to each partition in a structure. Player Eli Fisker [observed in 2011](#) that different energy levels in the various parts of a structure may create an imbalance, and suggested that a successful design should have an energy balance. This led to a concept of “*even energy distribution*”. The folding chain will seek an equilibrium, and uneven energy distributions create stress for that equilibrium across the partitions. Eli Fisker [posted a detailed article about this concept in 2014](#), and [commented further on it in 2016](#).

²⁴ Based on player Hoglahoo's “[Reverse Spore Walk-through](#)”.

²⁵ From “[Stack Caps](#)” in the Eterna Wiki.

²⁶ in the “[Advanced puzzle solving guide](#)”

²⁷ Based on player Hoglahoo's “[Reverse Spore Walk-through](#)”.

BOOST A LOOP

The last pair at the end of a stack is a closing pair. Beyond the stack and into a loop, the first set of unpaired pieces, one game piece on each side, can form a *terminal mismatch*. The mismatch pieces are ones that *do not* normally pair in Eterna. “A,G” and “G,A” and “U,U” are popular terminal mismatch position pieces for boosting.²⁸

Eli Fisher wrote, “As a general rule, the higher positive energy in a loop, the more potentially unstable it is. So what we want to achieve by boosting a loop is to lower its energy.”²⁹

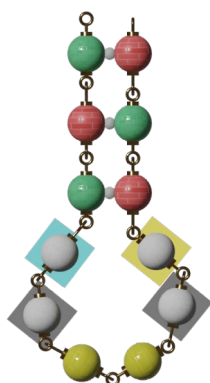
We could think of boosting a loop as reinforcing its closing pair, or as substituting for what could have been another pair inside the loop.

We cannot boost bulges, 3-piece hairpin loops, or certain multibranch loops (those having no unpaired pieces between their closing pairs).³⁰

28 From [the “Boosting” article](#) in the Eterna Wiki.

29 “[Another lesson in boost points](#)” section of Eli Fisker’s “Boosting” guide.

30 From [the “Boosting” article](#) in the Eterna Wiki.



Row	Pieces	Sequence	Loop energy	Total energy	Total change
1	CG, GC, CG, A, A, A, A, A, A	CGCAAAAAAGCG	+3.9	-1.9	
2	CG, GC, CG, U, A, A, A, A, A	CGCUAAAAAGCG	+3.7	-2.1	-0.2
3	CG, GC, CG, A, A, A, A, A, U	CGCAAAAAUGCG	+3.6	-2.2	-0.3
4	CG, GC, CG, G, A, A, A, A, A	CGCGAAAAAGCG	+3.2	-2.6	-0.7
5	CG, GC, CG, A, A, A, A, A, G	CGCAAAAAAGCG	+4.0	-1.8	+0.1
6	CG, GC, CG, C, A, A, A, A, A	CGCCAAAAAGCG	+4.4	-1.4	+0.5
7	CG, GC, CG, A, A, A, A, A, C	CGCAAAAAAGCG	+3.9	-1.9	0.0
8	CG, GC, CG, A, U, A, A, U, A	CGCAUAAUAGCG	+3.9	-1.9	0.0
9	CG, GC, CG, A, G, A, A, G, A	CGCAGAAAGAGCG	+3.9	-1.9	0.0
10	CG, GC, CG, A, C, A, A, C, A	CGCACAAACAGCG	+3.9	-1.9	0.0
11	CG, GC, CG, U, A, A, A, A, U	CGCUAAAAUGCG	+3.4	-2.4	-0.5
12	CG, GC, CG, G, A, A, A, A, G	CGCGAAAAAGCG	+3.8	-2.0	-0.1
13	CG, GC, CG, C, A, A, A, A, C	CGCCAAAAAGCG	+4.5	-1.3	+0.6
14	CG, GC, CG, U, A, A, A, A, G	CGCUAAAAGGCG	+3.6	-2.2	-0.3
15	CG, GC, CG, G, A, A, A, A, U	CGCGAAAAUGCG	+4.3	-1.5	+0.4
16	CG, GC, CG, U, A, A, A, A, C	CGCUAAAACGCG	+4.0	-1.8	+0.1
17	CG, GC, CG, C, A, A, A, A, U	CGCCAAAAUGCG	+4.6	-1.2	+0.7
18	CG, GC, CG, G, A, A, A, A, C	CGCGAAAACGCG	+3.4	-2.4	-0.5
19	CG, GC, CG, C, A, A, A, A, G	CGCCAAAAGGCG	+2.5	-3.3	-1.4

(((.....)))
1
123456789012

Figure 59. A hairpin loop with a variety of boosting attempts. Negative total changes are successful.

A simple structure with a 3- stack and a 6- hairpin loop can illustrate boosting. In the figure above, row 1 lists a solved sequence that fills the hairpin with As. The loop has an energy of +3.9, but the stack has an energy of -5.8, so the structure's total energy is -1.9 and it holds together in both target viewing mode and natural viewing mode. Positions 4 and 9 in the chain are the terminal mismatch positions.

In rows 2 through 7, we see what happens when we change a single piece in one terminal mismatch position. U is successful. G works on one side but not the other. C either goes the wrong way or does nothing.

Rows 8 through 10 show that changing the loop pieces *next to* the terminal mismatch positions has no effect on the loop energy.

In rows 11 through 19, we change both terminal mismatch pieces at the same time. Rows 11 through 13 change both positions to the same piece; and rows 14 through 19 alternate pieces in both positions.

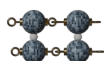
For this structure, a “C,G” terminal mismatch is the most effective boost by far. The next most effective is G,A followed by U,U and G,C. The remaining effective mismatches are A,U; U,A; U,G; and G,G. It's interesting that a G,A mismatch is very effective, but its reverse A,G mismatch is not. The ordering also makes a difference between U,G and G,U.

SWAP PAIRED PIECES

When we swap or flip a pair, we reverse the order of its game pieces. We swap a CG for a GC. We swap an AU for a UA. What we're actually doing is changing the identity of both game pieces at their individual positions in the chain, but it seems like we're just flipping the paired pieces around. Whatever we call it, though, swapping or flipping pairs changes stacking energy.³¹

A stack of length 2 shares an energy between its pairs. We can call this a *stacking energy*. This energy represents the attraction between the four game pieces as a set. It's as if the four pieces are forming a miniature loop. The more negative the energy, the stronger the attraction.

Rows in the following table list the 2-pair stack sets, the sequence of those four game pieces in a chain, and the stacking energy. The rows appear in order from strongest (most negative) to weakest (most positive) stacking energy.



Row	Pairs	Sequence	Energy
1	GC, CG	-GC-GC-	-3.4
2	CG, CG	-CC-GG-	-3.3
3	GC, GC	-GG-CC-	-3.3
4	GU, CG	-GC-GU-	-2.5
5	GC, UG	-GU-GC-	-2.5
6	UA, CG	-UC-GA-	-2.4
7	CG, GC	-CG-CG-	-2.4
8	GC, AU	-GA-UC-	-2.4
9	AU, CG	-AC-GU-	-2.2
10	GC, UA	-GU-AC-	-2.2
11	GU, GC	-GG-CU-	-2.1
12	UA, GC	-UG-CA-	-2.1
13	AU, GC	-AG-CU-	-2.1
14	CG, UG	-CU-GG-	-2.1
15	CG, UA	-CU-AG-	-2.1
16	CG, AU	-CA-UG-	-2.1
17	UG, CG	-UC-GG-	-1.5
18	GC, GU	-GG-UC-	-1.5
19	UG, GC	-UG-CG-	-1.4
20	CG, GU	-CG-UG-	-1.4
21	AU, UG	-AU-GU-	-1.4
22	GU, UA	-GU-UA-	-1.4
23	UA, UG	-UU-GA-	-1.3
24	GU, AU	-GA-UU-	-1.3
25	UA, AU	-UA-UA-	-1.3
26	AU, UA	-AU-AU-	-1.1
27	UA, GU	-UG-UA-	-1.0
28	UG, AU	-UA-UG-	-1.0
29	UA, UA	-UU-AA-	-0.9
30	AU, AU	-AA-UU-	-0.9
31	AU, GU	-AG-UU-	-0.6
32	UG, UA	-UU-AG-	-0.6
33	GU, GU	-GG-UU-	-0.5
34	UG, UG	-UU-GG-	-0.5
35	UG, GU	-UG-UG-	+0.3
36	GU, UG	-GU-GU-	+1.3

Table 2. Stacking energies of paired pieces.

Compare the top three rows in the table. Only GC and CG pairs appear in these rows. Notice how a single swap, from a GC to a CG, takes the energy from -3.4 to -3.3. Another single swap, though, from

³¹ Reference to “flipping” comes from the “[Base Pair Flipping](#)” article in the Eterna Wiki.

a CG in row 2 to a GC in row 7, takes the energy from -3.3 to -2.4. Row 7 happens to have the weakest stacking energy among sets of GC and CG pairs.

Compare the bottom four rows in the table. Only UG and GU pairs appear in these rows. Notice how the bottom two rows have positive numbers, and the next two rows have negative numbers. The weakest stacking energy is +1.3, for a GU,UG set. Since it is a positive number, it works to destabilize this portion of a stack. This can be a handy feature, for some game scenarios.

Now compare row 36 (a GU,UG set) with row 4 (a GU,CG set). Changing a single game piece takes the stacking energy from +1.3 to -2.5. That's a dramatic change!

AU appears from row 8 to row 31. UA appears from row 6 to row 32. GC appears from row 1 to row 19. CG appears from row 1 to row 20. UG appears from row 5 to row 36. GU appears from row 4 to row 36. These ranges give the general idea that AU and UA are "medium" or "stabilizing", GC and CG are "strong" or "more stabilizing", and UG and GU are "weak" or "more destabilizing"; but the ranges actually overlap significantly, so context matters.

BALANCE THE GAME PIECES

There is a tactic to place GC and CG pairs throughout a chain, hoping to quickly match the natural mode structure to the target mode structure. A variation of the tactic places AU and UA pairs throughout the chain first, then places GC and CG pairs to assure the matching modes. In either case, after placing the GC and CG pairs, we can work to balance the game pieces: individually replace GC and CG pairs with AU and UA pairs and view the resulting mode match; then individually replace GC, CG, AU or UA pairs with UG and GU pairs and view the result, until reaching the desired state of balance among the pairs.

Player Brouard suggested³² working from the ends of the structure toward the middle, when replacing GC and CG pairs. The ends would be hairpin loops, and the middle would likely be a multibranch loop. An external loop could be considered an “end” if it has one closing pair, or could be considered a “middle” if it has multiple closing pairs.

Player Brouard also suggested³³ spreading UG and GU pairs away from each other, and if that is not possible, placing them so that the Gs and Us line up with each other. We can understand this idea in the “Swap paired pieces” table, above, where rows 33 and 34 show UG,UG and GU,GU sets having energy value -0.5; but row 35 shows UG,GU having +0.3 and row 36 shows GU,UG having +1.3.

A chain sequence having a greater chance for success in the laboratory would likely have 50% to 70% GC and CG pairs, 50% to 30% AU and UA pairs, and some UG and GU pairs if the chain is supposed to interact with other things.

32 on a slide in the “Beginner’s Guide to Puzzle Solving”.

33 on the “Ciliate telomerase - Level 1” slide and the “Solving the Puzzle: Adding G-U” slide

FIND ALTERNATE SOLUTIONS

There are two interpretations of alternate solutions.

In the one interpretation, we've solved the puzzle already, and are now interested in additional solutions differing in their characteristics.

When we have a puzzle solution, it may be interesting to test the stability of that solution. One way to do this is to proceed through the structure, swapping a single pair back and forth as we go. After trying a single pair at a time, we could try swapping two adjacent pairs at a time. We are purposely trying to stress the original puzzle solution, to see what it does under that stress.

In the other interpretation, we're trying to solve a difficult puzzle and want to experiment with alternative approaches. This means we return to the early game stage, repeatedly, until happy with the resulting game play.

APTAMERS

An aptamer³⁴ is an internal loop whose game pieces will bind to an outside object if that object is present. The aptamer acts as a docking port for the outside object.³⁵

Find FMN aptamer puzzles at [<https://eternagame.org/puzzles?search=fmn>].

Find puzzles with “aptamer” in their title at [<https://eternagame.org/puzzles?search=aptamer>].

See the “[FMN aptamers](#)” [lab page](#) for a summary of Eterna’s early involvement.

SWITCHES

A switch is a puzzle that wants a single chain sequence to fold into two or more stable structures depending on whether an outside influence is present or absent. There are tutorials in Eterna for switch puzzles, as well as sets of switch player puzzles.

One approach to a switch puzzle is to solve the unbound state (the state without the outside object present) first, since the unbound state has to be solved anyway. Then, modify your sequence bit by bit until it allows the bound shape.³⁶

One of the basic tactics to solve switches is to make parts of the structure less stable. That is, adjust game pieces in switching areas (parts of the structure that should move during the switch) so that energy values become more positive.

Player ElNando888 suggested³⁷ to skip boosting loops as a first step in solving switch puzzles, reasoning that the boost can interfere with the switching action. ElNando888 also suggested working from the more difficult parts of the structure toward the simpler parts of the structure.

34 defined in the “[Aptamers](#)” section of “[Lab guide for new players](#)”

35 from “[Switch puzzle guide](#)”

36 “[Switch puzzle guide](#)”

37 in “[Switch Training Series 1.1 Walkthrough](#)”

A characteristic of switch puzzles is the energy bonus given by the outside molecule or object. This means we only have to arrange the sequence for our bound target structure so that it is within the bonus energy value above the unbound state's solution energy. For example, if the unbound state solution sequence has an energy of -30, and the outside molecule has a bonus of -4.86, we must arrange our bound state target sequence to have an energy between -30 and -25.14.³⁸ We can summarize the energy bonus characteristic with a relationship between energies:

bound structure with molecule < unbound structure < bound structure without molecule

Our chain will fold first into the unbound structure ("OFF"); and when the outside molecule shows up, the chain will grab the molecule and fold into the bound structure ("ON").

Eterna's "[Switch Tutorial - lesson 3l: Training Wheels](#)" puzzle description has commentary that ends with this: "In summary, this lesson has shown that large loops make a switch easier to solve because the freeloading {pieces} can be mutated without affecting the energy in the loops. A good strategy for solving switches is to start with boosting the loops and follow the pair runs that these choices lead to. Choosing GC pairs for a little less than half the total pairs make a switch stable and also flexible enough to change between the shapes."

38 "[Switch puzzle guide](#)"

MASTERING THE GAME

DO TUTORIALS

The [Eterna home page](#) has a “Complete your lab” section. This is the main tutorial series. The first set of puzzles is “[Nucleotide Mixer](#)”. The following sets are “Gene Synthesizer”, “Thermocycler”, “Next-Generation Sequencer”, “RNA Polymerase”, “Microfluidic Chip”, “Fluorescent Probes”, “Laser Microscope”, “Liquid Robotics”, and “Supercomputer”. It often helps to read the descriptions on each puzzle page, to learn more while working through the progression.

Player Zama designed “[[Tutorial](#)][[Scripted](#)] [Fun Boost Tutorial aka Turketorial](#)” to teach common boosting techniques for a variety of loops.

Player jnicol designed “[Fun with John](#)” as an informal set of switch tutorials. The introduction is in the “[Switch Tutorial - lesson 1: Introducing the Molecule](#)” puzzle description.

There is [a set of free form switch tutorials](#). Another [set of switch tutorial puzzles](#) and [a third set](#) are also available.

Eli Fisker designed [an “Evolution of switch” tutorial puzzle set](#).

Eli Fisker wrote [another set of lab tutorial puzzles](#). Eli and Omei wrote [switch lab tutorial puzzles](#).

Player jandersonlee wrote a [flavin mononucleotide \(FMN\) tutorial puzzle](#).

SOLVE MORE PUZZLES

Eterna has many puzzle series. In general, the descriptions accompanying these puzzles can be informative.

Challenge puzzles intend to help us practice our skills. In Eterna, navigate to the “Puzzles” page, then filter for “Challenge” and “Uncleared”. (Or, go directly to [<https://eternagame.org/puzzles?filters=challenge,notcleared>].) The description for each puzzle introduces some of the science behind that puzzle, as a bonus.

Puzzles made just for fun appear in a puzzle table at [<https://eternagame.org/puzzles?search=Fun>]. These puzzles have “fun” in their titles.

The “Eterna 100” is a set of puzzles at [<https://eternagame.org/puzzles?search=Eterna100>] that the robots generally have trouble solving.

Switch puzzles are at [<https://eternagame.org/puzzles?search=switch>].

Player puzzles in general are at [<https://eternagame.org/puzzles?filters=player>].

In general, we can enter some text from puzzle titles into the search box, to narrow the selection of puzzles. (For example, write “3 states” without the quotes to see a table of 3-state switch puzzles.)

LEARN THE SCIENCE

Eterna's [challenge puzzle](#) descriptions often introduce the science behind the puzzle. This is a topical way to learn the science.

[“The scientist and the riboswitches”](#) was an Eterna blog item on 2014-05-13.

Read articles written by Eterna researchers and players:

[“RNA design rules from a massive open laboratory”](#), published in 2014.

[“Principles for predicting RNA secondary structure design difficulty”](#), published in 2016.

Also see [“Appendix A: the Science”](#)!

BROWSE LAB RESULTS

Browsing lab results can give clues for developing new game tactics.

Player Eli Fisker wrote [“Reading the lab results”](#) probably in 2014.

STUDY CAMPAIGNS

Eterna players conduct experimental lab campaigns from time to time. We can become better players by studying these campaigns.

We can find information about the campaigns in news items, blogs, and in the lab project pages. From the Eterna website home page, we can navigate to “News”, where we can browse through news items and blog entries. We can also navigate to “Play > Labs” at [<https://eternagame.org/labs>], where we can browse and search through lab project pages. On the pages where we can search, we can use search phrases to filter for a particular campaign. The search phrase is exact, with no wildcards; and search results can have the phrase in their title or their content.

By searching in the lab and by browsing through the news items, we can learn about the campaigns. Here is a selection of search phrases:

[A]/[B]	Neck strength analysis
Classic Eterna	OpenCRISPR
Cloud Lab	OpenTB
Eterna History Tour	Puzzle Progression
FMN Aptamer	Ribosome Challenge
FMN Switch	Ribosome Pilot
Kissing Loop	Riboswitches On Chip
Light-up	RNA in - RNA out
Lightning Round	SHAPE Profile
Logic Gate	Tetraloop Competition
miRNA Switch	Thermodynamic Mimics
Multistate RNA	

Table 3. Phrases to search for game campaigns in Eterna.

Incidentally, we can make up search phrases to find information on other topics of interest in the Eterna website. Just remember that the search phrase is exact — no wildcards.

DESIGN PUZZLES

We can design puzzles in Eterna. Making puzzle designs is a good way to gain further understanding and experience in how the game works. It can also be fun for other players to solve our own puzzles!

According to Eli Fisker's "Tips and trick guide" ("Switch puzzles" section), ElNando888 wrote "[How to create FMN switch puzzles](#)". According to the same "Tips and trick guide" section, jnicol wrote "[How to Create a Switch Puzzle as a Cloud Lab Candidate](#)".

TOSS THE RULES

Rules are fine for cooperative play. Sometimes, though, we discover more by tossing the rules and going wild. Especially, tossing this guide's rules can lead to something new.

APPENDIX A: THE SCIENCE

WILL THE REAL GAME PIECES PLEASE STAND UP?³⁹

“A” is for *adenine*, a small chemical compound having 5 carbon atoms, 5 nitrogen atoms, and 5 hydrogen atoms. The compound is shaped like two rings fused together and laying flat side by side, with a short spur from one ring. The rings are carbon and nitrogen, and the spur is a nitrogen. The hydrogen atoms stick out like little antennae from two of the carbons and two of the nitrogens. We can learn much more about adenine at <https://en.wikipedia.org/wiki/Adenine> and <https://pubchem.ncbi.nlm.nih.gov/compound/adenine> .

“U” is for *uracil*, a small chemical compound having 4 carbon atoms, 2 nitrogen atoms, 2 oxygen atoms, and 4 hydrogen atoms. The compound is shaped like a single flat ring with two spurs. The ring has all the carbon and nitrogen atoms, and the spurs are the oxygen atoms. The hydrogen atoms stick out like little antennae from two of the carbons and both of the nitrogens. Articles at <https://en.wikipedia.org/wiki/Uracil> and <https://pubchem.ncbi.nlm.nih.gov/compound/uracil> tell more about uracil.

“G” is for *guanine*, a small chemical compound having 5 carbon atoms, 5 nitrogen atoms, 1 oxygen atom, and 5 hydrogen atoms. Guanine is shaped much like adenine, but with two spurs. One spur is nitrogen, and the other is oxygen. The hydrogen atoms stick out like little antennae from one of the carbons and three of the nitrogens. We can find more information about guanine at <https://en.wikipedia.org/wiki/Guanine> and <https://pubchem.ncbi.nlm.nih.gov/compound/guanine> .

“C” is for *cytosine*, a small chemical compound having 4 carbon atoms, 3 nitrogen atoms, 1 oxygen atom, and 5 hydrogen atoms. Cytosine is shaped much like uracil, except that one of the spurs is a nitrogen. The hydrogen atoms stick out like little antennae from two of the carbons and two of the nitrogens. The <https://en.wikipedia.org/wiki/Cytosine> and <https://pubchem.ncbi.nlm.nih.gov/compound/cytosine> pages tell us more about cytosine.

Adenine, uracil, guanine, and cytosine are classified as [nucleobases](#) and [heterocyclic compounds](#).

39 We give a nod to “To Tell the Truth” and another to a great [episode of “The Twilight Zone”](#).

SEQUENCE

Two additional chemical compounds are a part of our topic.

Ribose is a small chemical compound having 5 carbon atoms, 5 oxygen atoms, and 10 hydrogen atoms. Here, it is shaped like a pentagon with spurs. The ring starts with an oxygen by itself (no spur), then goes around to four carbons. Three of these carbons have a spur of oxygen and hydrogen. The fourth carbon branches from the ring to a fifth carbon and then oxygen and hydrogen. Each of the pentagon ring's carbons has a hydrogen antenna, and the fifth carbon has two hydrogen antennae. More information about ribose is at <https://en.wikipedia.org/wiki/Ribose> and <https://pubchem.ncbi.nlm.nih.gov/compound/5779> . (The form of ribose we're using here is more specifically named β -d-ribofuranose.)

Phosphoric acid is a small chemical compound having 1 phosphorus atom, 4 oxygen atoms, and 3 hydrogen atoms. It is shaped as a tetrahedron with the phosphorus in the center and the oxygen atoms at each tip of the pyramid. Three of the oxygen atoms have a hydrogen antenna. The variant of phosphoric acid we're using here is named *dihydrogen phosphate* because it has only two of the three hydrogen atoms. More information appears at https://en.wikipedia.org/wiki/Phosphoric_acid , <https://pubchem.ncbi.nlm.nih.gov/compound/1004> , <https://en.wikipedia.org/wiki/Phosphate> , and <https://pubchem.ncbi.nlm.nih.gov/compound/1003> .

A nucleobase attaches through one of its nitrogen atoms to a ribose by kicking off one of the oxygen-hydrogen spurs on the ribose.⁴⁰ In turn, a ribose loses the oxygen-hydrogen spur from its carbon-carbon-oxygen branch to allow its fifth carbon to attach to an oxygen in the dihydrogen phosphate. This nucleobase, ribose, and phosphate molecule is a *ribonucleotide*, or more generally a *nucleotide*.

The ribonucleotides are the basic building blocks of *ribonucleic acid* (RNA). Each nucleotide is a link in the RNA chain. To build the chain, a second nucleotide attaches through another of its phosphate oxygen atoms to the first nucleotide's third ribose carbon. We read or write the RNA sequence by starting at the first nucleotide's fifth ribose carbon, then moving through its third ribose carbon to the second nucleotide, and likewise to the third nucleotide and onward.⁴¹

We can think of the linked phosphate and ribose compounds as a backbone for the RNA sequence, and we can consider the nucleobases to be the distinguishing part of each link in the RNA chain.

40 The combination of nucleobase and ribose is a *nucleoside*. The act of combining is more complex than stated here!

41 We read from the "five prime end" (5' end) to the "three prime end" (3' end). See [directionality in molecular biology](#).

PAIRING

Even after forming a sequence in an RNA chain, nucleobases still attract each other. At the end opposite the ribose, a nucleobase has exposed hydrogen and nitrogen and oxygen atoms in some combination. If the geometries are right and there isn't too much interference from nearby atoms, *hydrogen bonding* can take place, and two nucleobases form a *base pair*.⁴²

Adenine's end exposes a set of hydrogen, nitrogen, and hydrogen atoms.

Uracil's end exposes a set of oxygen, hydrogen, and oxygen atoms.

Guanine's end exposes an oxygen, hydrogen, hydrogen atom set.

Cytosine's end exposes a hydrogen, nitrogen, oxygen atom set.

If adenine and uracil are available, their geometries allow two hydrogen bonds. Adenine's central exposed nitrogen and uracil's central hydrogen atom bond together. One of adenine's exposed hydrogen atoms, extended by a nitrogen spur, bonds with one of uracil's exposed oxygen atoms.

Uracil and guanine can also form two hydrogen bonds, but at a somewhat different angle. In this case, uracil's centrally exposed hydrogen bonds with guanine's exposed oxygen, and guanine's centrally exposed hydrogen bonds with an oxygen exposed in uracil.

Guanine and cytosine match well enough to form three hydrogen bonds. The side oxygen on guanine bonds with the side hydrogen on cytosine. The side oxygen on cytosine bonds with the side hydrogen on guanine. And, the central hydrogen on guanine bonds with the central nitrogen on cytosine.

These hydrogen bonds and the base pairs they form along the RNA chain support further levels of structure.

⁴² Further reading:

["Base pair"](#) in Eterna Wiki.

["Hydrogen bond"](#) and ["Intermolecular force"](#) in Wikipedia.

["Hydrogen bond"](#), ["Hydrogen bond in theoretical organic chemistry"](#), and ["Definition of the hydrogen bond \(IUPAC Recommendations 2011\)"](#) by the International Union of Pure and Applied Chemistry (IUPAC).

["Hydrogen Bonds"](#) and ["Hydrogen Bonding in Water"](#) by Martin Chaplin.

["Intermolecular bonding — hydrogen bonds"](#) by Jim Clark.

STRUCTURE

We started with the chemical compounds and their atoms. Let's move our perspective to the structural levels. We can think about an RNA molecule at four levels of structure.⁴³

The first level is a one-dimensional line. It is the RNA sequence, written as the initials of the ribonucleotides in “five prime to three prime” order. This is the fine jewelry chain we used as a metaphor early in this guide.

The second level is two-dimensional, built by fastening together segments of the RNA chain, using the hydrogen bonds of base pairs. At this level, the Eterna game screen shows us two-dimensional diagrams from target viewing mode and natural viewing mode, and we work with the motifs resulting from the base pairing.

The third level is the RNA molecule in three dimensions. At this level, a long stack we saw in two dimensions now becomes a helix⁴⁴. We realize that the RNA chain folds on itself in multiple angles and directions, bringing distant motifs into closer quarters.

In the fourth structural level, the RNA molecule interacts with other molecules. This is where an RNA molecule might be, for instance, part of a spliceosome in a nucleus or part of a ribosome participating in protein synthesis.⁴⁵

43 Further reading:

“[Biomolecular structure](#)” in Wikipedia.

44 See a [short Eterna Wiki video](#) illustrating the formation of a helix from paired bases.

45 See, for instance, a [seven minute video](#) on transcription, translation, and protein synthesis for digestion.

ENERGY

The basic goal in an Eterna puzzle is to arrange the game pieces (nucleotides) so that their RNA chain folds into the structure we want. To help us, the game screen shows us a number of energy values: “total” energy, “natural/target delta” energy, and the “loop” or “stack” energy. There is an energy stat between each set of two pairs in a stack. There is an energy stat for each loop. There is an overall energy stat for an entire chain or structure.

Where do these energy stats come from? They come from molecular modeling — in other words, from a simulation of reality.

Atoms vibrate. Electrons shift and fluctuate. Bonds between atoms stretch and bend and rotate. Electric charges attract and repel. All these actions involve energy. A large molecule such as RNA becomes a challenge to model exactly, so we simplify with approximations and averages.⁴⁶

What we're interested in is the energy that is available (“free”) to drive the chemical reactions that make or break our RNA structure. We're interested in *Gibbs free energy*. We find it through a combination of the total system energy (enthalpy), temperature, and disorder (entropy).⁴⁷

Eterna uses functions and parameters within a set of molecular models to calculate the Gibbs free energy changes and the energy stats we see in game. Eterna uses the [ViennaRNA Package](#) from the Institute for Theoretical Chemistry at Universität Wien, Austria. Eterna also uses the [Nearest Neighbor Database](#) from the Department of Biochemistry & Biophysics at the University of Rochester Medical Center, Rochester, New York.⁴⁸

Think of a stack of length 2. Two adjacent nucleotides in an RNA sequence are joined together by covalent bonds. Two adjacent nucleotides further along in the sequence, also joined together by covalent bonds, join these first two nucleotides using hydrogen bonds. This gives a basically rectangular shape made with the four nucleotides as vertices, the covalent bonds as two opposing edges, and the hydrogen bonds as the other two opposing edges. The nearest neighbor method

46 Take the deep dive into “[Introduction to Macromolecular Simulation](#)” by Peter Steinbach, 2019, [Center for Molecular Modeling](#), National Institutes of Health, Bethesda, Maryland.

47 Further reading and viewing:

“[Chemical reaction: Thermodynamics](#)” and “[Gibbs free energy](#)” in Wikipedia.

“[Chemical bond](#)”, “[Hydrophobic effect](#)”, “[Molecular modelling](#)” in Wikipedia.

“[The Laws of Thermodynamics, Entropy, and Gibbs Free Energy](#)”, “[Gibbs Free Energy](#)”, “[Using Gibbs Free Energy](#)”, “[Gibbs Free Energy and the Relationship between Delta G, Delta H, and Delta S](#)” in YouTube.

48 Further reading:

“[Nucleic acid thermodynamics: nearest neighbor method](#)” in Wikipedia.

“[NNDB: the nearest neighbor parameter database for predicting stability of nucleic acid secondary structure](#)”, 2009 research article.

“[Testing the nearest neighbor model for canonical RNA base pairs: revision of GU parameters](#)”, 2012 research article.

“[A unified view of polymer, dumbbell, and oligonucleotide DNA nearest-neighbor thermodynamics](#)”, 1998 research article.

determines the change in free energy among these nucleotides and bonds, and gives the energy values appearing in “Swap paired pieces”.

The nearest neighbor method can also determine the change in free energy involved when two of the four nucleotides are mismatched, as in a closing pair and terminal mismatch situation. (There are no hydrogen bonds to make the fourth edge of the rectangle.) This plays a part in “Boost a loop”.

The Nearest Neighbor Database also determines changes in free energy for internal loops, bulges, hairpin loops, multibranch loops, and external loops.⁴⁹

These molecular models are the basis for the energy values seen in the Eterna game screen. The exact values may differ in a given situation in game.

49 See the NNDB [tutorial index page](#).

APPENDIX B: THE ART

We use our eyes and our minds not only to see but to comprehend. We learn from informative graphs and diagrams and illustrations. We describe impressively informative art with words like “illuminating” and “visionary”.

The figures and tables in [“History of molecular theory”](#) illustrate how concepts of molecules changed over time. [“Molecular model”](#) discusses and shows some of the techniques used in physical representations today. [“Molecular graphics”](#) has a few more figures and much more detailed discussion, and leads to several other articles on related topics.

Eric Westhof gave presentations in [2010](#) and [2011](#) on visualization techniques to represent RNA molecule structures. Yann Ponty reviewed RNA visualization in [a 2012 presentation](#). Paul Gardner presented RNA visualization challenges [in 2013](#). Jeffrey Heer gave [a keynote address on data visualization principles in 2014](#). Some of the concepts reappeared in printed form in the [“Visualization of biomedical data” review article](#) in 2018.

David Goodsell gave [a presentation in 2014 on making science beautiful](#).

RCSB Protein Data Bank's [PDB-101 portal](#) includes a [guide to understanding molecular graphics programs](#), a [guide to ligands and their graphic representations](#), and a [“Molecules of Life: Nucleic Acids” gallery](#), as well as these pieces of art:

- [“Adenine riboswitch in action”](#)
- [“Fluorescent RNA aptamers”](#)
- [“Ribosomal subunits” \(rotating GIFs\)](#)
- [“Ribosome”](#)
- [“Riboswitches”](#)
- [“RNA polymerase”](#)
- [“Self-splicing RNA”](#)
- [“Small interfering RNA \(siRNA\)”](#)
- [“Transfer RNA”](#)
- [“Transfer-messenger RNA” \(tmRNA\)](#)

We can use molecular graphics software to create our own visualizations of macromolecules, including RNA. Links to such software appear in [an RCSB PDB list](#) and in [a list in Wikipedia](#). Here are a few examples having galleries or web interfaces.

- [Chimera](#) is a molecular modeling system with [an image gallery](#) and [an animation gallery](#).
- [Jmol](#), a viewer for chemical structures, has [a gallery of images](#).
- [RNAComposer](#) is an RNA modeling server. Enter an RNA sequence and optionally a secondary structure in dot-bracket notation. Then, wait a minute for the program to produce output including a rotating model.
- [Rosetta](#) is a molecule modeling suite with a server called [ROSIE](#). It has several RNA modeling protocols.

For the “Structure of an active human histone pre-mRNA 3’-end processing machinery” research article in February 2020, the [online supplementary material](#) includes two animated movie visualizations of the process.

Here are some videos with artistic presentation examples.

“[The RNA Enigma](#)” (3:33) uses a popular graphic arts approach. The first half of the video describes RNA chains and structures and functions. The second half describes Eterna as of 2014!

“[RNA Folding | HHMI BioInteractive Video](#)” (0:32) transforms an RNA sequence through a secondary structure (three hairpins, a multibranch loop, a stack, and an external loop) and into a three dimensional space filling model.

“[HIV Life Cycle | HHMI BioInteractive Video](#)” (4:56) illustrates the entire replication process of a virus, including depictions of viral RNA, messenger RNA, and an RNA-enzyme-protein complex.

“[Where Did Life Come From?](#)” (13:39) includes graphics fitting DNA, RNA, and proteins into a system diagram (6:46), graphics describing RNA (7:17) and ribozymes (7:47 and 8:28), and diagrams of nucleobases in molecules (8:33).

“[RNA interference \(RNAi\): by Nature Video](#)” (5:06) uses computer animation techniques as of 2011 to depict siRNA, microRNA and RNA processes.

“[Gene Silencing Methods: CRISPR vs. TALENs vs. RNAi](#)” (13:00) uses graphic arts techniques and includes depictions of sgRNA (2:07), a ribonucleoprotein (2:16), RNAi (3:43), siRNA (3:50), and target mRNA (4:03). These depictions appear through the remainder of the video.

“[Positive-sense Single-stranded RNA \(\(+\)ssRNA\) Virus – RNA Virus Genomes – COVID-19 | Lecturio](#)” (6:44) uses a simple but visually effective motif and graphic technique to distinguish RNA in a 2020 lecture presentation.

“[Coronavirus SARS-CoV-2 structure](#)” (2:16) uses molecular modeling and computer graphics as of 2020 to portray the virus. A short RNA close-up animation begins near (1:18).

“[Generation and action of siRNAs and miRNAs](#)” (6:49) uses a combination of graphic arts techniques and computer graphics as of 2014 to illustrate the topics. It includes an animation of RNA folding near (0:48). By contrast, “[V. Narry Kim \(IBS and SNU\) 1: microRNA Biogenesis and Regulation](#)” (26:07) and its sequel, “[V. Narry Kim \(IBS and SNU\) 2: Tailing in the Regulation of microRNA and Beyond](#)” (20:34), use a different presentation style, with graphic arts and some computer graphics techniques as of 2017, for covering the same subject area.

Khan Academy uses its distinctive presentation style in a 2015 video, “[Molecular structure of RNA | Macromolecules | Biology | Khan Academy](#)” (11:29), to detail the chemical compound difference between DNA and RNA. Amoeba Sisters use a very different presentation style in a 2019 video, “[DNA vs RNA \(Updated\)](#)” (6:30), to describe other differences between DNA and RNA.

Some more variety in presentation styles and graphic techniques:

- “[mRNA, tRNA, and rRNA function | Types of RNA](#)” (2:08) by 2 Minute Classroom in 2019.
- “[Anna Marie Pyle \(Yale U./HHMI\) Part 1: RNA Structure](#)” (23:03) by iBiology in 2014.
- “[What is RNA | Genetics | Biology | FuseSchool](#)” (2:55) by FuseSchool in 2017.
- “[Structure Of Nucleic Acids - Structure Of DNA - Structure Of RNA - DNA Structure And RNA Structure](#)” (3:51) by Whats Up Dude in 2017.
- “[Nucleic Acids: DNA and RNA](#)” (7:04) by Professor Dave Explains in 2016.

Finally, “[Can Scientists Use RNA to Create a Coronavirus Vaccine? | NOVA | PBS](#)” (5:28) uses a combination of graphic arts, video arts, and computer graphics techniques as of 2020, as background in the video. (A scientist involved with Eterna, Rhiju Das, is one of the interviewees in the video.)

APPENDIX C: THE PATRONS

We would like to publicly recognize and thank the following patrons who supported the production of this guide:

Marilyn Y.

Chris B.

Eileen W.

Elizabeth M.

Stanley J.

Monica M.

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Tim H.

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