Challenge Puzzle Solution Guide

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Revision #	Description of Change
1.0	Initial Release
1.1	Update and fix typos
2.0	Complete revision with inclusion of theory's and examples

1. Definitions

Word \ Abbreviation	Definition
X-1, X-2, X-3	The X represents the classic variable x found in mathematics representing all natural whole numbers.
Segment	A segment is defined as an end loop (the tail counts as an "end loop" in this instance) and the connecting stacks including any structural change in it leading up to the larger base loops that have 3 or more total segments connected to them.
Nts	Nucleotide
Legs	Complete segment from base loop to end loop regardless how many branches a leg has
Rod	A sequence of stacks that forms a "solid" line of nucleotides from one loop to another.

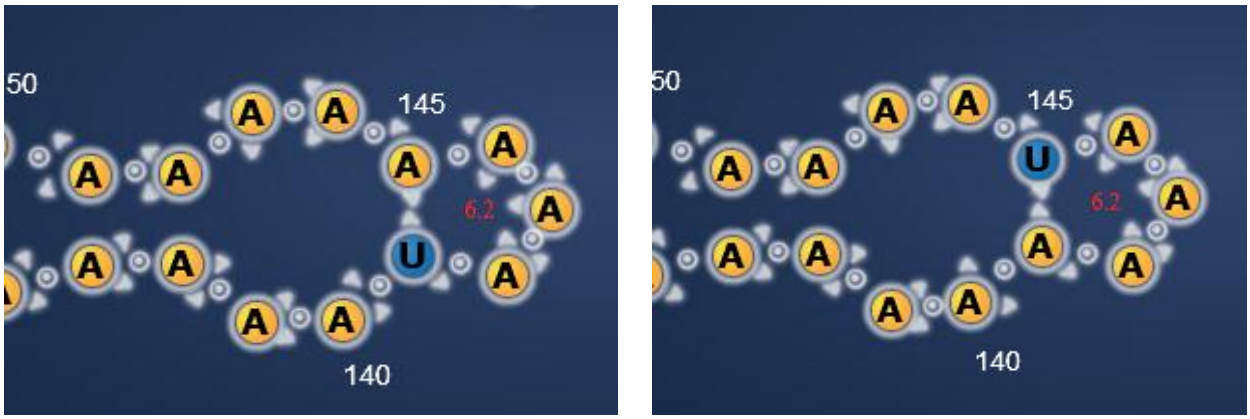
2. Initial Design and Stabilization

RNA should not be considered a single solid structure but a multitude of small segments that are interconnected to each other in such a way that when pulled apart form a single large segment. I believe that this approach will help alleviate difficulty in conveying the essence of mutating specific nucleotides (nts) of RNA in such a way that it begins to pull itself in on itself. Think of this as a critical mass that must be reached in order to break free from the straight line generated by the complete AA pair stacks.

This can be achieved by either a small amount of very strong pulling nucleotide pairs (GC, CG) or a large amount of medium strength nucleotide pairs (AU, UA). The weakest forming bond pairs (GU, UG) are not so good for providing main structural support as they are for re-enforcement of the main structure. An example would be if a large free standing wall was to be leaning so far in one direction as to require structural re-enforcement you would use some sort of pole like structure to prop up the wall and prevent further collapse. You would not build a wall out of it but it sure can help with other stuff.

The best method I have found for building this wall is to use a series of alternating AU, GU nucleotides throughout all the stacks as the initial groundwork. The main requirement of any stable structure is a solid foundation and any bumps or holes along the path will produce a defective product with a potential sinkhole at its center. However, a stable structure prevents a sinkhole event resulting in the once almost perfectly stable structure from being ripped apart and returning to its original stick shape or close to it.

The method for accomplishing this is to first important thing to keep in mind is to find all the end loops and place a single AU pair at the base of each end loop ensuring that the blue Uracile nucleotide is on the lowest numbered nts. Refer to Figure 1 and two for examples.



Good Bad

Figure 1

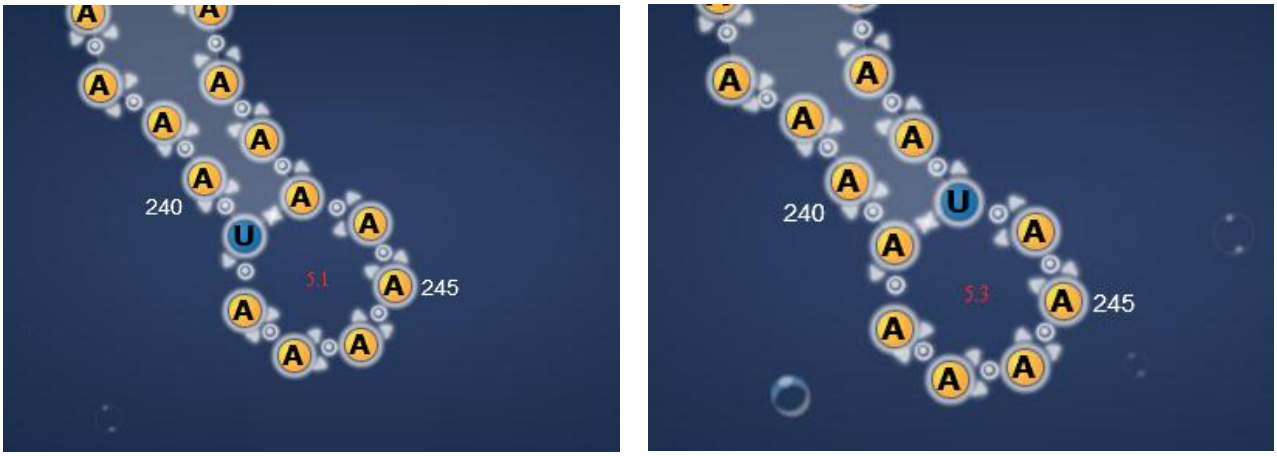


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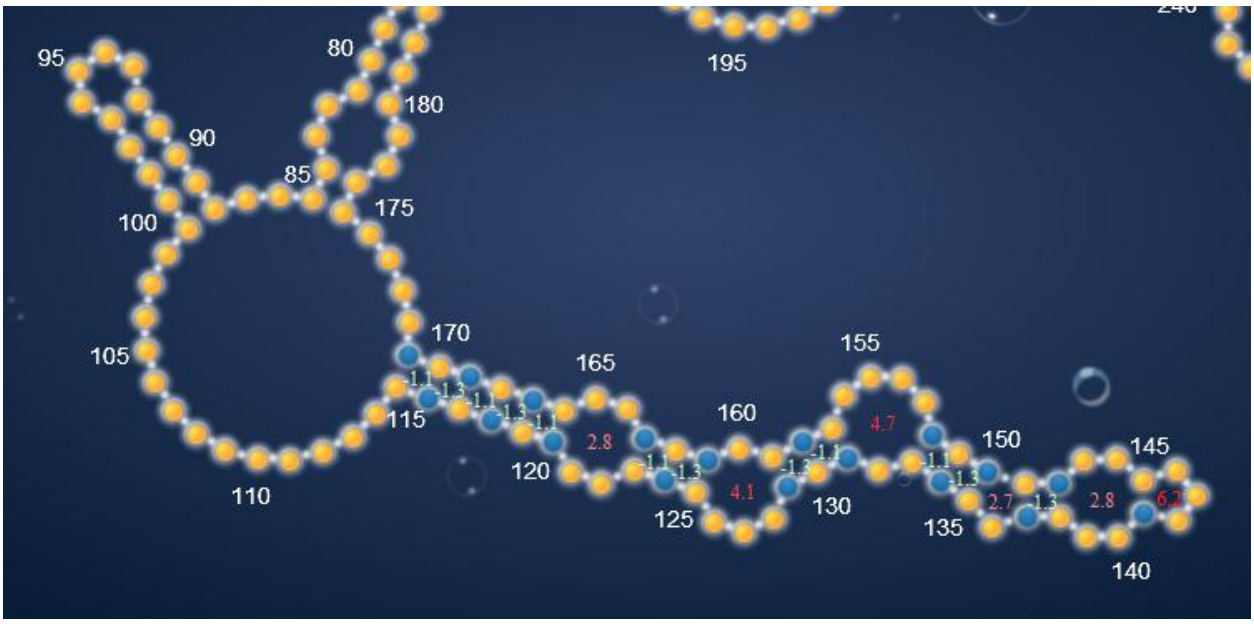
Figure 2

Next, you want to lay out a pattern of alternating AU, UA pairs using the AU pairs placed in Figures 1 and 2 as the starting reference point to base the alternating sequence on. When a loop is reached continue the pattern on the other side always starting the first pair on a stack after a loop with the blue Uracile nucleotide on the lowest numbers nts as in figures 1 and 2. This is done to achieve a consistent pattern that is easily repeatable and consistently strong and due to all the segments having the same starting pattern and they are offset in one way or another this variation within the design is the randomization factor instead of relying on a randomization algorithm which can be prone to issues. This process can also be tweaked to account for a lack of segment shape randomness as well. Refer to figures 3 and 4 for examples.



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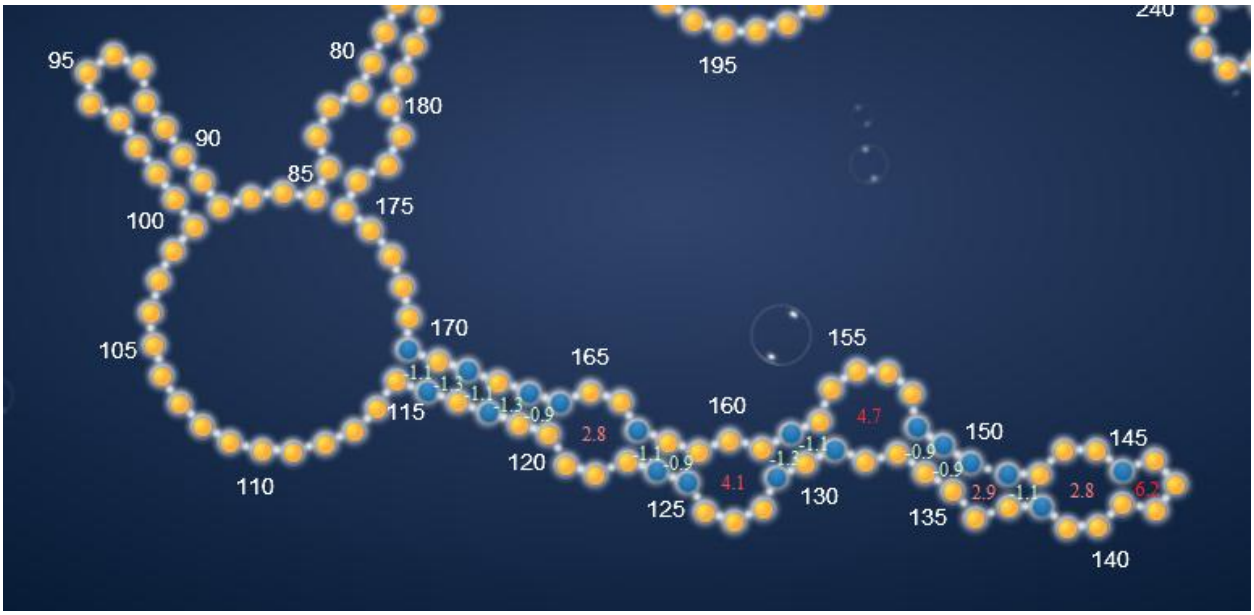

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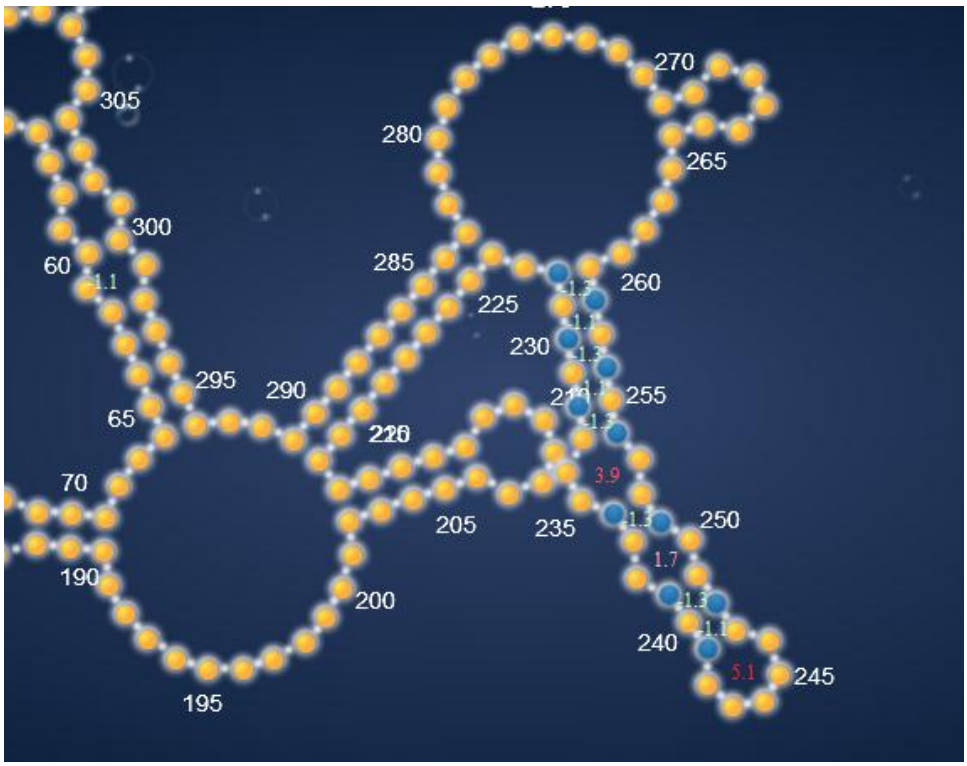
Figure 3



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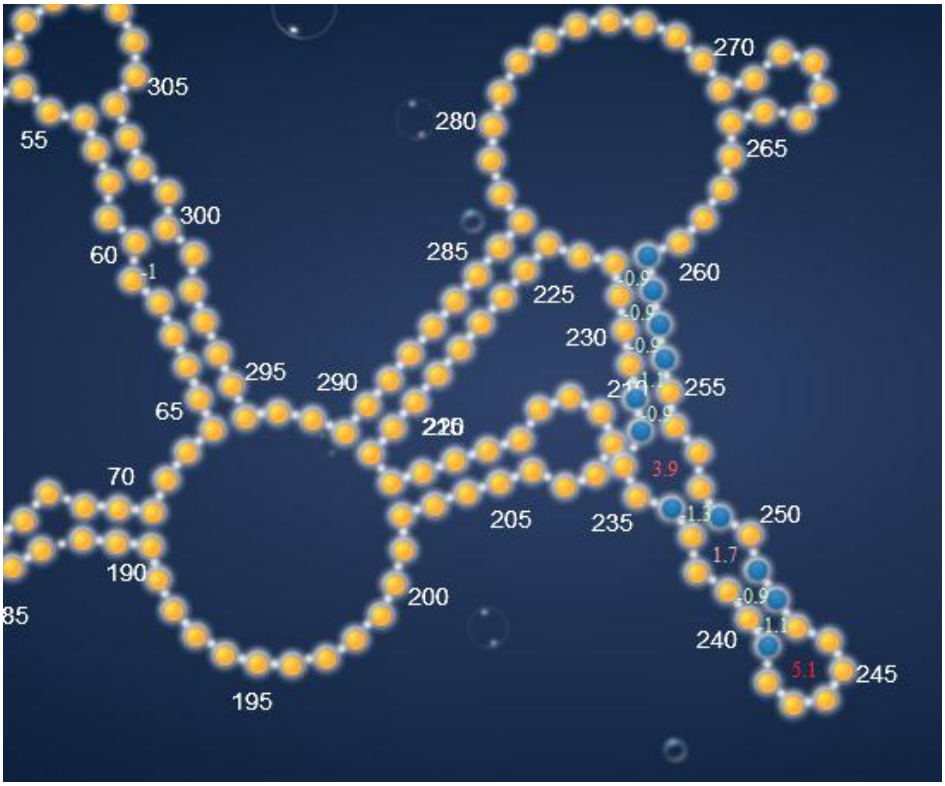


Bad

Figure 4

Once all the AU ground work is placed the support structures for the wall must be established. These support structures are established by boosting loops with red Guanine nucleotides in a way that decreases the positive bad energy and increases the negative good energy. This may sound weird but the larger the negative free energy the more solid a structure is. This may be because all the nucleotides are exerting such a strong consistent force on each other that they neither pull nor push away but if a nucleotide with a greater negative free energy in placed in the loop at specific spots it creates an imbalance that allows the negative free energy to affect each other without a penalty.

2.1 Special Loops

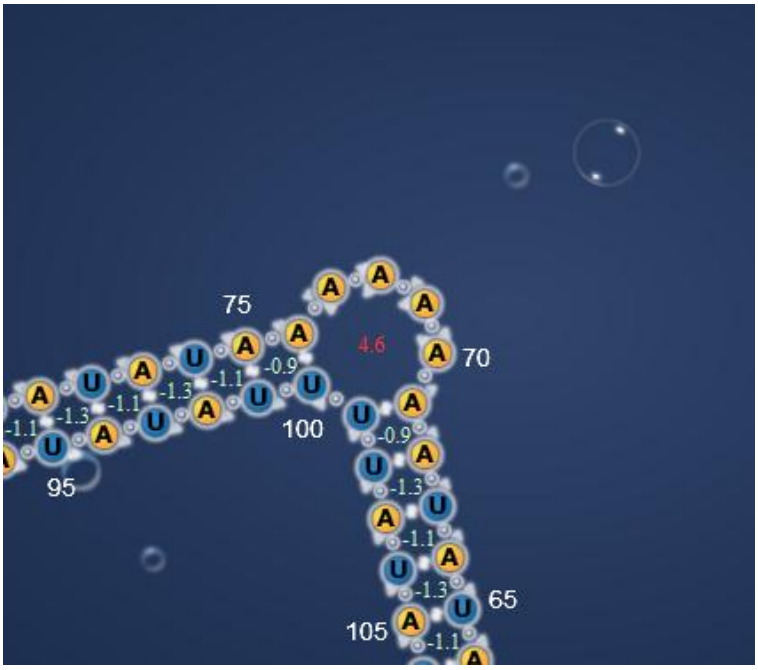
The first step is to search for any 0-X loops and change the stacks before and after the bulge formed by the X number of nucleotides to side by side AU or UA pairs depending on bend orientation to make them have the same orientation. Ensure that the A's are on the side away from the bulge and the U's are on the side with the bulge. Refer to Figures 5 and 6 examples.

Good

Bad

Figure 5

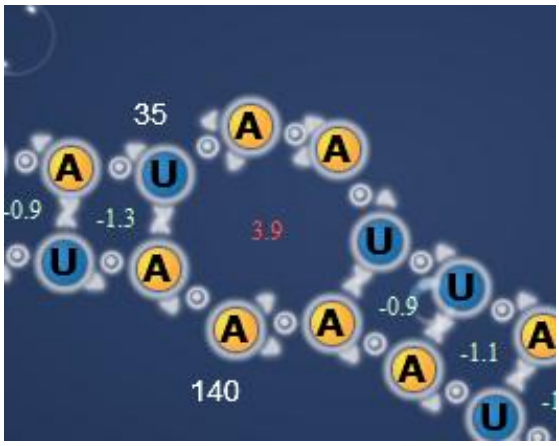
Good



Bad

Figure 6

Next, perform the same procedure on the 1-X loops placing AU or UA pairs at the beginning and end of the loop positioning them such that the A's are on the same side and the U's are on the same side. Ensure that the A's are on the inside of the bend formed and that the U's are on the outside of the bend formed. Refer to figure 7.



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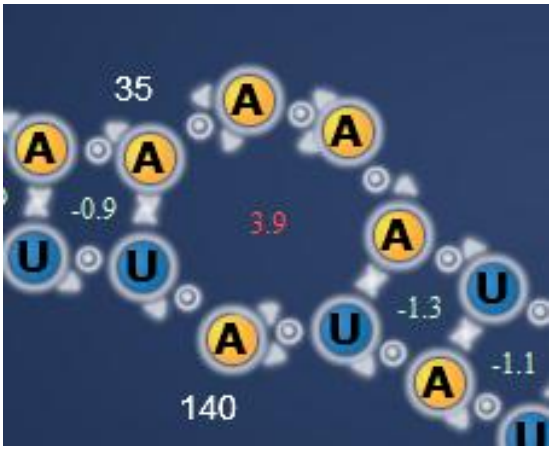

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Figure 7

2.2 Boosting

Boosting should be done in a sequence of steps to ensure the most efficient and effective use of red Guanine nucleotide is used. This is due to an excess amount of guanine often leading to an imbalance and sinkholes.

The following is the sequence of steps to take in outline form to ensure efficient boosting use:

a. Place a single red G at the nucleotide that is one number greater than the location of the blue U placed at the beginning of all the end loops earlier in the document.

Good Bad

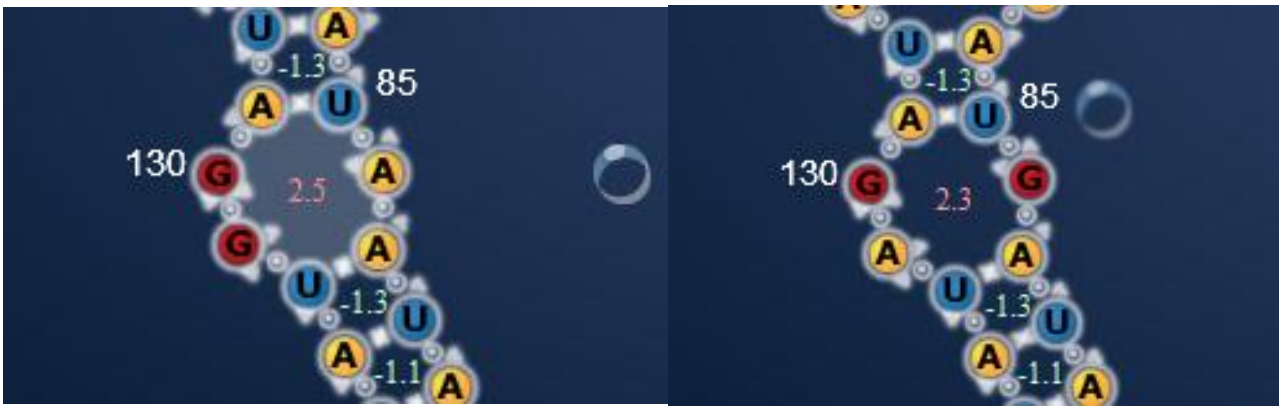
Figure 8

b. Search for any 1-1 loops and change the two nucleotides that bulge out to G.

Good Bad

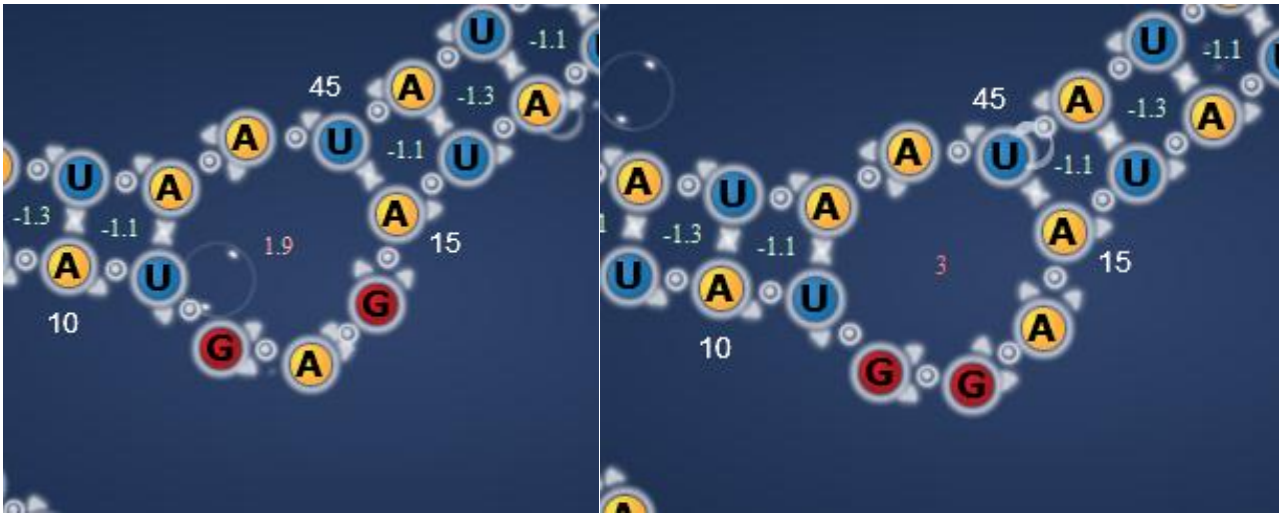
Figure 9

c. Find any 1-X loops (where X is greater than or equal to 2) and place two G's on the side with the X number of nucleotides. These G's should be placed such that they are immediately before and after the AU pairs in the connecting stacks



Good Bad

Figure 10



Good Bad

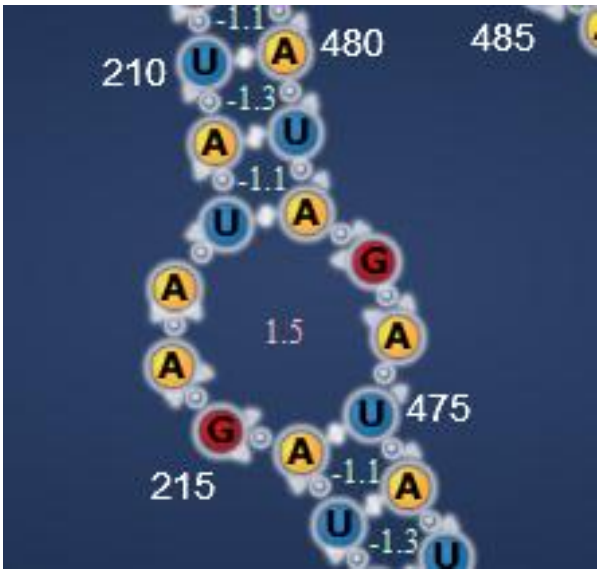
Figure 11

d. Find any X-X loops $where\ X \geq 2$ that are not base loops and place one G on each side off the loop with the G being placed on the highest numbered nucleotide of that side for that loop for a total of 2 G's in the loop.

Good

Bad

Figure 12

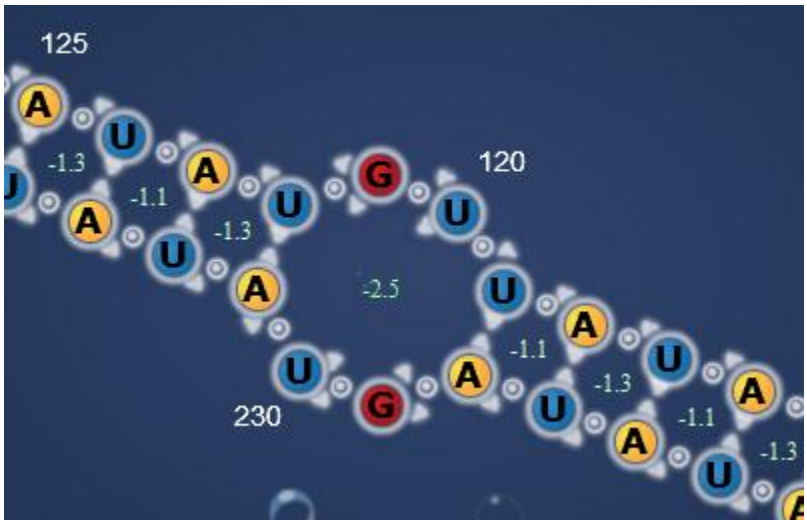


Good

Bad

Figure 13

e. Find any 2-2 loops and replace the two remaining yellow Adenine nucleotides (one on each side of the bulge next to a red Guanine placed earlier) with a two blue Uracil nucleotides, one on each side. When placing these two blue U's there may be a nucleotide mismatch that will cause the loop to lose its bonds. This will be covered in the next section, how to adjust for unwanted nucleotide mismatch.



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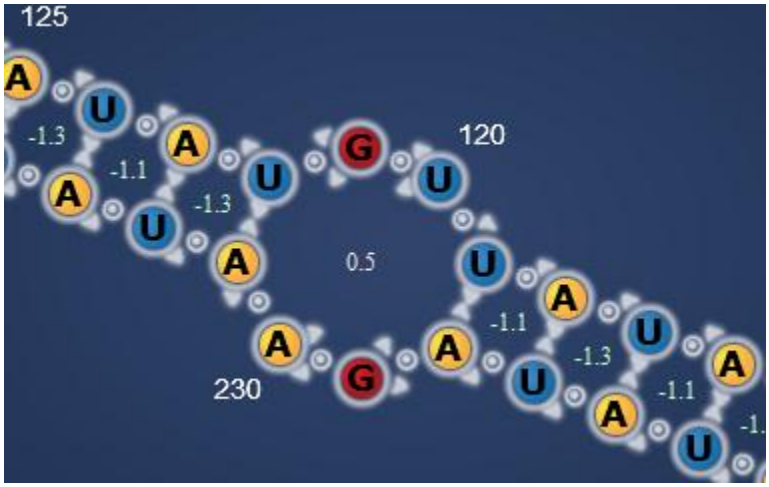
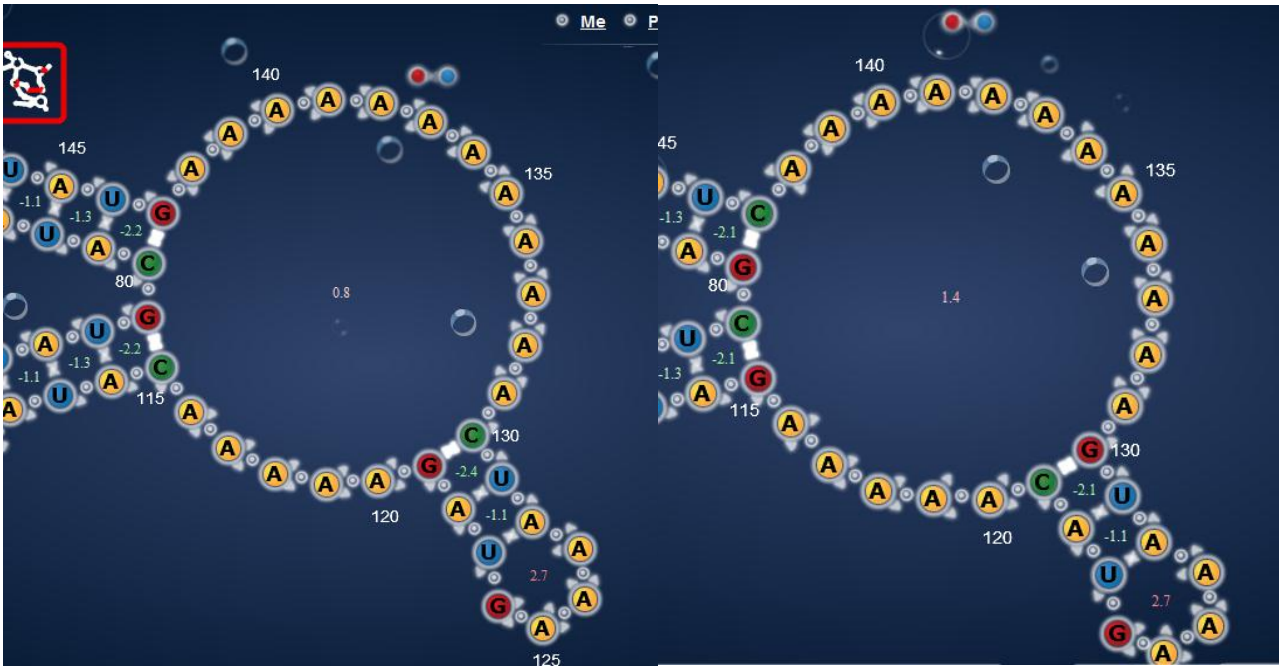


Bad

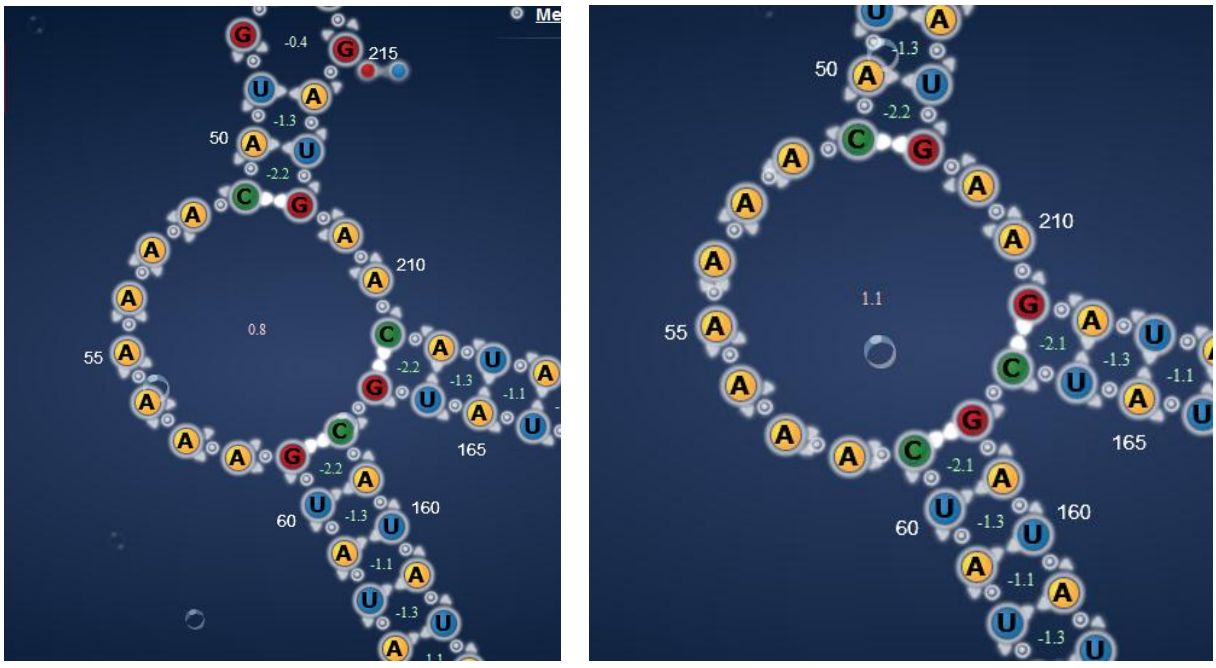
Figure 14

f. Search for any base loops and place a GC at each point a segment connects to a base loop. Ensure the G is placed in such a way as that the leading edge of the GC pair if it was traveling in a clockwise motion would be the red G.



Good Bad

Figure 15



Good Bad

Figure 16

At this point the support structures of the wall have been established and now it is time to lay the brick and mortar. Think of each loop and\ or set of stacks that has been stabilized as a brick stacked on the wall and the transitional area between loop and stack or loop and loop can be thought of as the mortar. This is due to the characteristic of the loop often bonding successfully, however due to a weakness in the stack adjoining the loop is pulled apart. This can be corrected by adjusting the positioning or laying down some mortar to help support the brick while it sets.

3. How to Adjust for Unwanted Nucleotide Mismatch

At this point the process becomes in depth and is no longer paint by color so to speak. This portion of the document will explain how to read the mini-map and interpret the red\white color changes\zones and how to compensate for any abnormalities.

The mini-map is one of the little boxes found in the top left corner of the puzzle screen all the way to the right of all the other boxes (See Figure 15) and this map correlates to the actual puzzle (Figure 16).

Figure 17

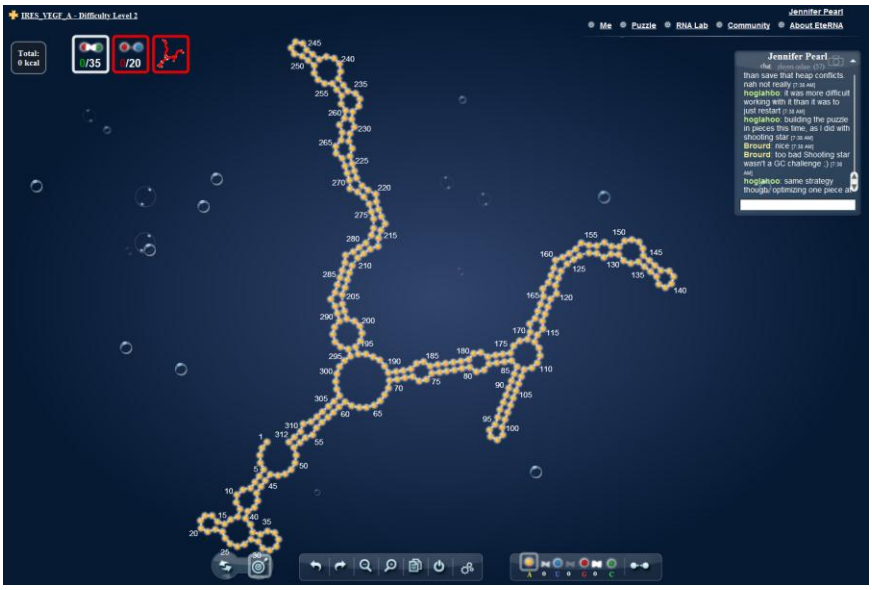


Figure 18

The sections of the puzzle that are not solved or successfully bonded in such a way to achieve the desired pattern are displayed as red and those that are solved or are successfully bonded in a way to achieve the desired pattern. Figure 17 shows the top segment solved and the bottom two segments unsolved. If you hover your mouse over the mini-map and perform a single click the window will expand for greater detail (Figure 18). This is a very important thing to know how to use.

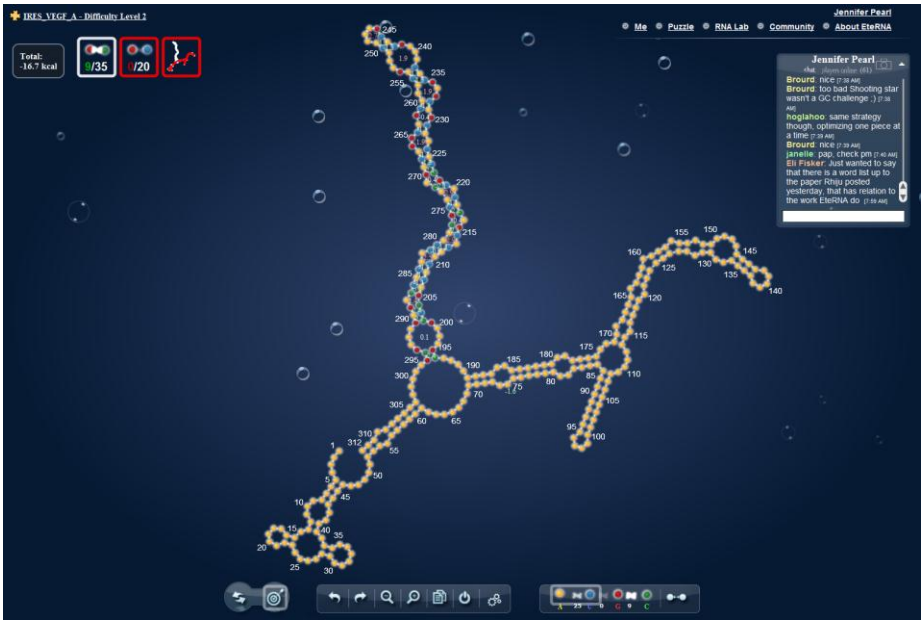


Figure 19

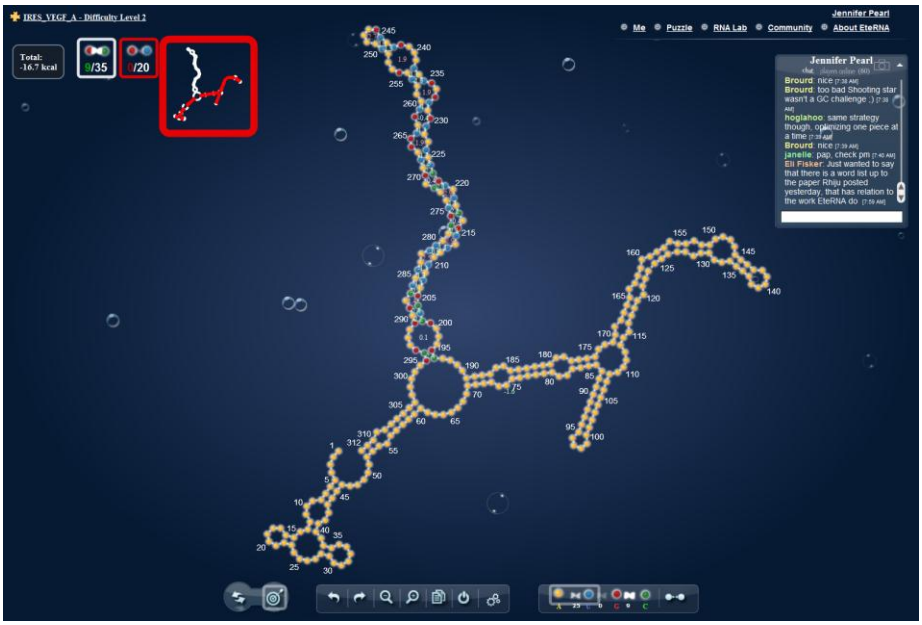


Figure 20

The goal now is to use this mini-map to help determine the best course of action to correct a problem area. I will be using the following challenge puzzle design.

Puzzle name: IRES_VEGF_A - Difficulty Level 2
Shape Notation: ....((((...((.((.....))....((......)).))..))))....(((.((((((.......((((..((((((..((((((((((((((....)))))))))....((.((((.((((((((.((..((((.....))))....)).)).))).))).))))).))..))))..))))))..))))....((....((((((((((((.((.(((.((((((.((.((...(((....(((....)))...))).)).)).)..)))).))))))))))).)))))).)))....))... ..))))))).)))
RNA Sequence: AAAGAUAUAAGAUAAUGAAAAAUAAAAAUGAAAAAAUAAUAGAUAUAAAAGAUAGUAUAUAAAAAAAAAUA UAGAUAUAUUGAUAUUAUAUAUAUGAAAAUAUAUAUAAAAAAAUGAUAUGAUAAUAAUGAUAGAUAUGAAA AAUAUAAAGAUGAUAUAUAUAUGAUAUGAUAAAUAUAGAUAUAUAGAUAUAAAAAUAAAGAUAAUAUAAA UAUUAUAUAUAAUAUAAUGAUGAGUAUAAAGUAUGAAAAUAAAGAUAAAUGAUGGAUAUAUAAUAAAAUU AUAUAUAUAUAAAGAUAAAAAAUAUAUAGUAU

Table 1

Using the data from Table 1 above you can either open that puzzle in the challenge puzzle section or build it in the puzzle maker if you wish to follow along on your computer at home. This puzzle solved up to this point in the guide should look like the example in figure 21.

Figure 21

If you zoom in on the section just to the right of the large base loop you will see a set of 2-2 loops (Figure 22). These 2-2 loops are not binding correctly and let us see how we know this.

Figure 22

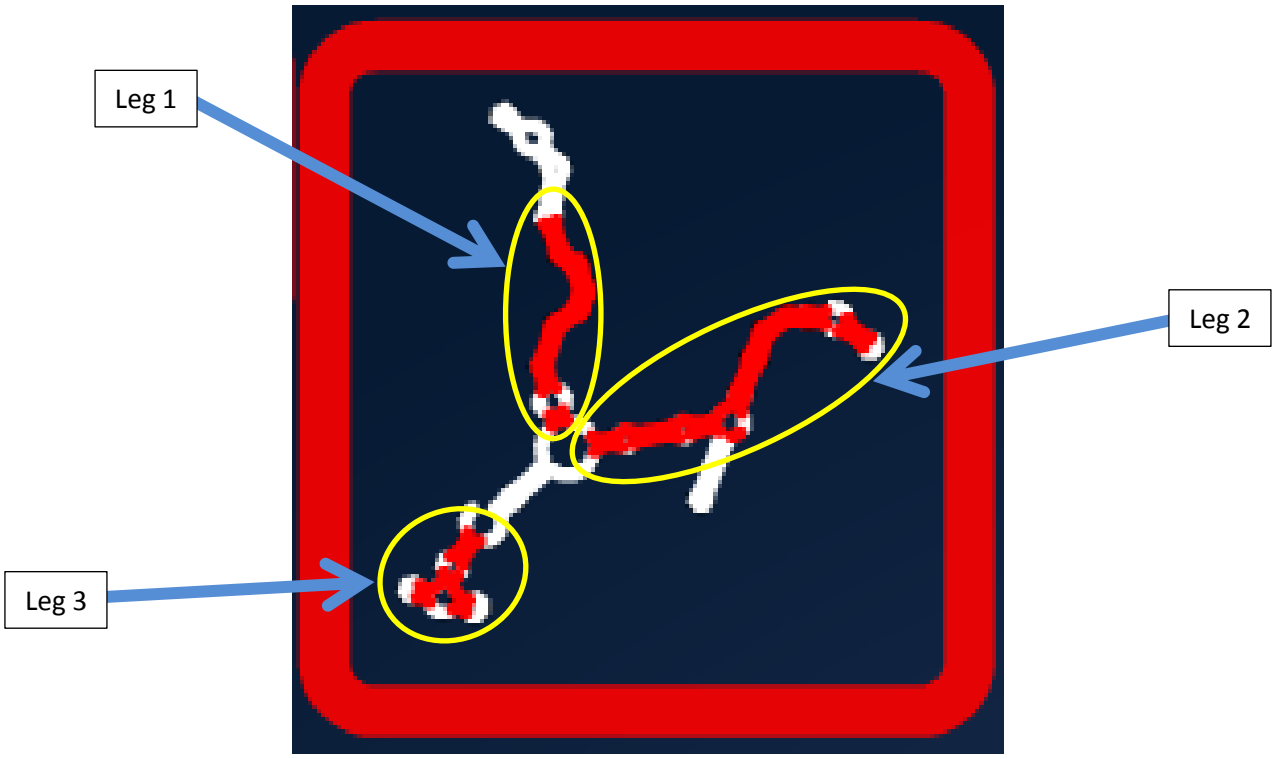
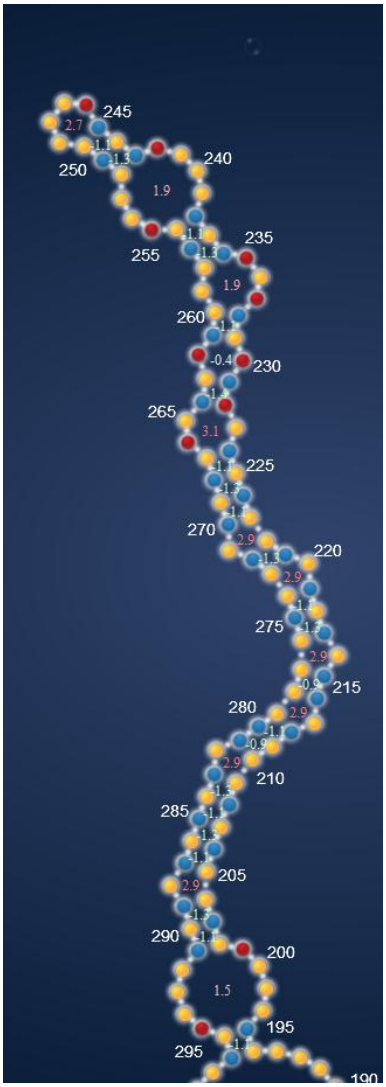


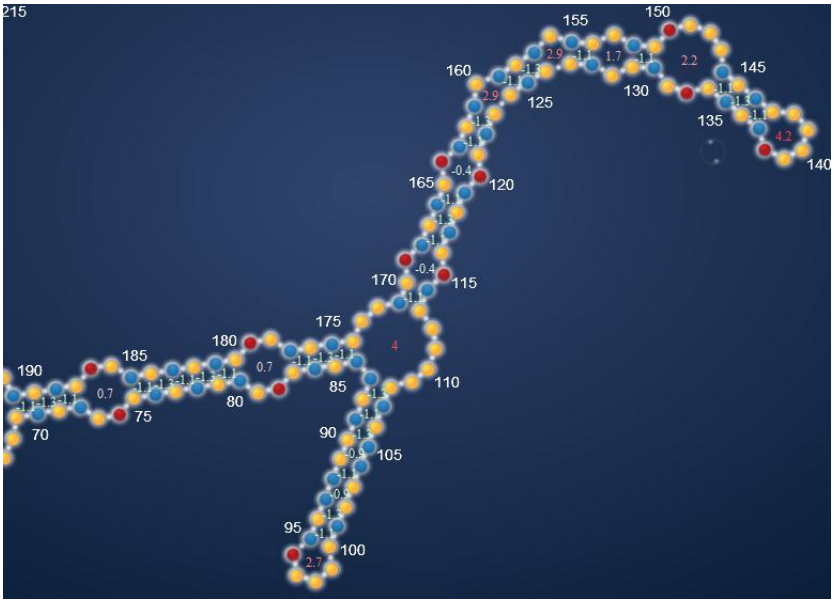
Figure 23

Looking at Figure 22 and Figure 23 there are a lot of things to learn about this design. This design has 3 main legs and 2 of those legs have severe structural integrity issues and the 3rd only has minor issues. Legs 1 and 2 have the major problems (structural integrity) and leg 3 has the minor problem. I use the term major problem to describe when the structural integrity issues extend into a base loop so as that the main body of the segment does not bond. This can be applied to each successive layer of the design as we fine tune our adjustment. A minor problem would be used to describe a segment whose main body is structurally sound yet the end loop portion of the segment is having issues. These are often fixed by correcting the major problems first so it would be inefficient to approach the minor problem first. Thus, major problem areas are investigated first.

Looking at Figure 24 which is a side to side comparison of legs 1 and 2 we see that the problem area in leg 1 is 0-1 loops and the problem areas in leg 2 is 2-2 loop's and 0-1 loops. 0-1 loops are tricky and can change based on anything however 2-2 loops are pretty straight forward and these are sitting a set of straight stacks that once stabilized will provide a significant amount of structural integrity to the entire puzzle (not solve it completely just help a lot) without the use of GC pairs. That being said the focus for now will be leg 2 and I say for now simply because as soon as one thing is stabilized often another can become destabilized.



Leg 1



Leg 2

Figure 24

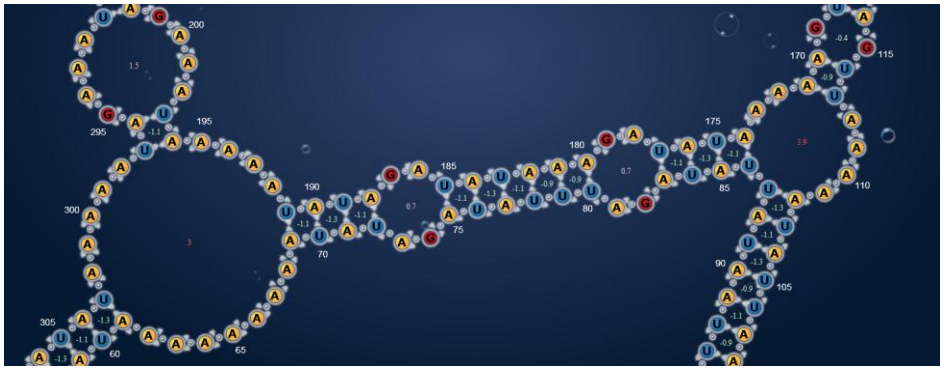


Figure 25

Zooming in on the design now with leg 2 as the focus with particular interest in the 2-2 loops we see that the stacks connecting the 2-2 loops are connected to two base loops (Figure 25) forming what I am calling a rod that behaves a lot like a compression bolt. Imagine the stacks being hollow rods and the loops the mounting hardware required to make the compression bolt functional. A compression bolt is a type of bolt that is used to provide support to structures to prevent them from coming apart without interfering with the objects intended purpose. A type of compression bolt that his relevant to the current metaphor of a wall is the type that many people see every day on the side of older building generally made of brick that look like little stars. The little stars, squares, or circles you see with bolt looking things are there to hold the building together due to structural instability. Currently the rod is not functioning properly and we need to help a form proper bond and to do this we must move to the next step in the loop boosting phase, the 2-2 loop GU mismatch.

Zoom out one last time and switch back and forth between Natural and Target mode and watch how the RNA fold splits evenly at the “compression” rod in leg 2. Watch as the upper section separates and forms a cup type shape that it maintains briefly before the forces from the other unstable sections interact with it creating conflicting forces forcing a complete breakdown or sinkhole. Figure 25 is a screenshot of the compression rod in leg 2 just as it separates when shifting from Target to Natural mode. Now we know exactly what it is we are correcting let’s fix the compression rod.

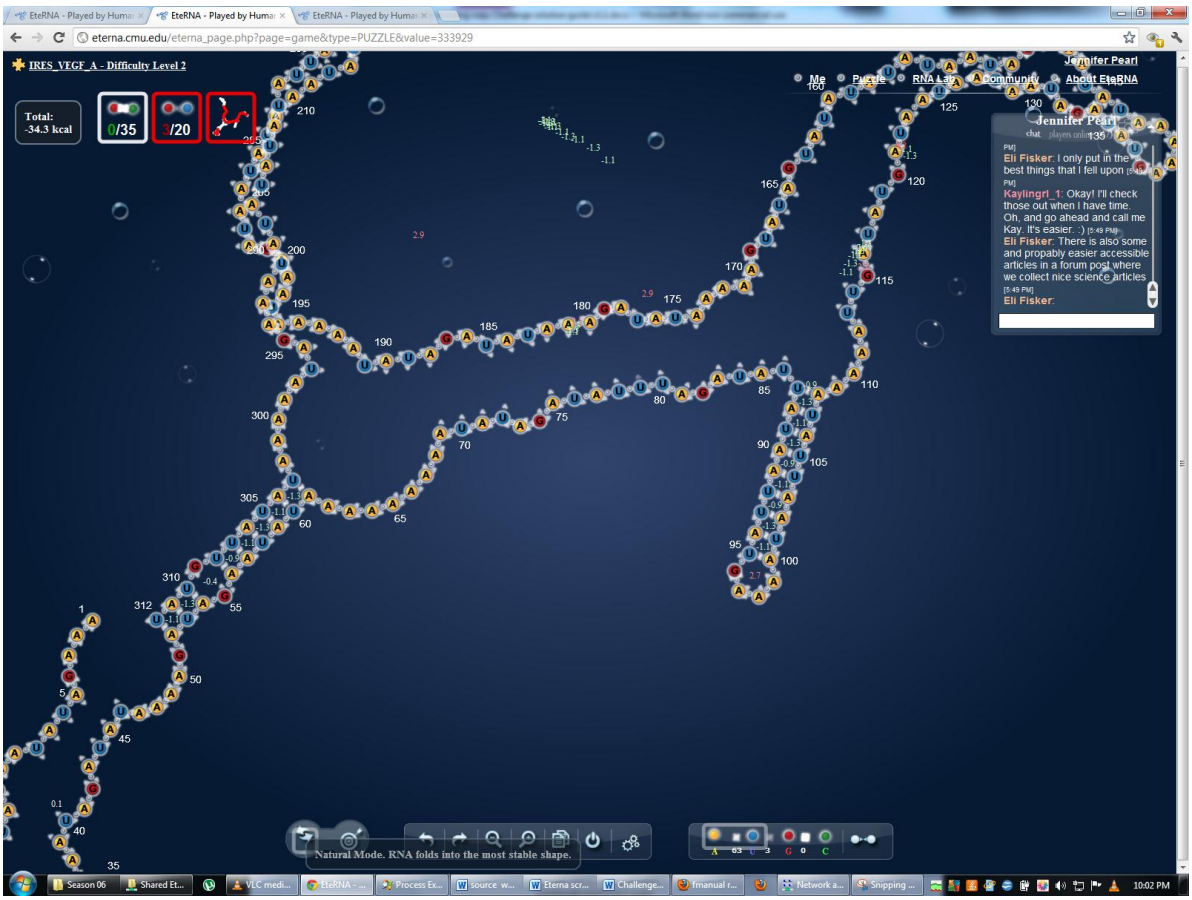
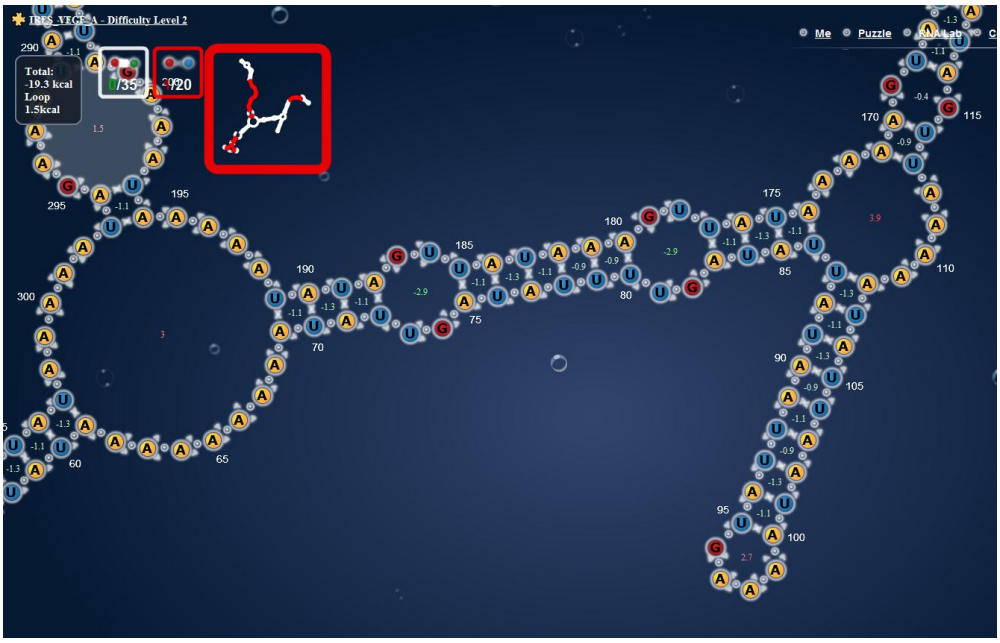
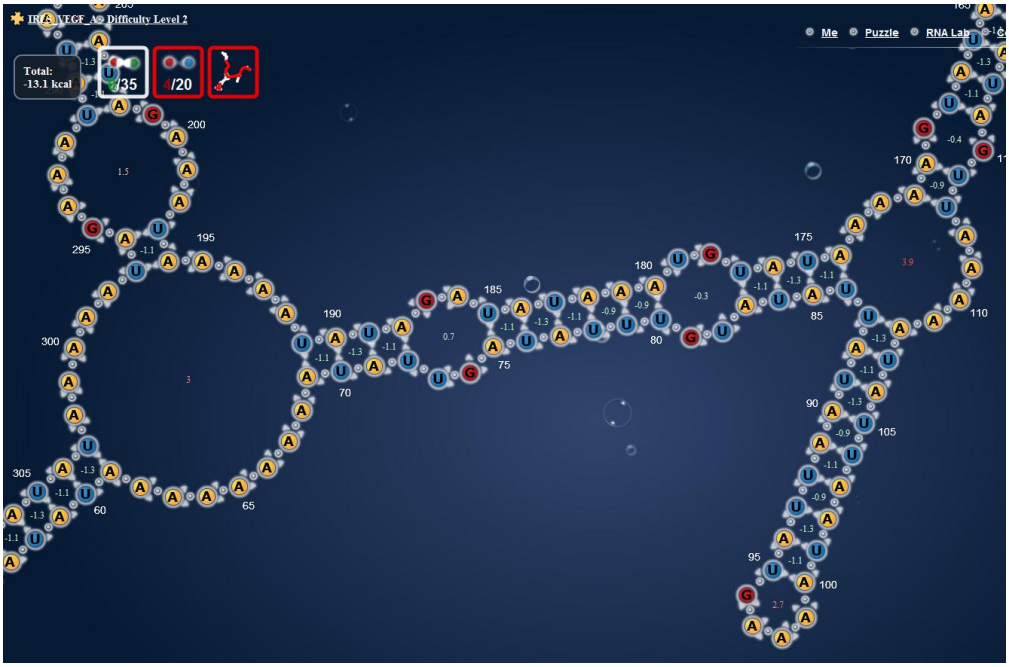


Figure 26

Switch back to Target mode and place a total of two blue Uracile nucleotides in the 2-2 loops where the yellow Adenine nucleotides are at but do not touch the red Guanine nucleotides placed earlier for boosting. Refer to Figure 27 for example. If you play around with the AU pairs alongside the 2-2 loops the design stability will change. I encourage you to play around with this and there are some great references available by many top players here is the link http://eterna.cmu.edu/eterna_page.php?page=newsitem&nid=51904.



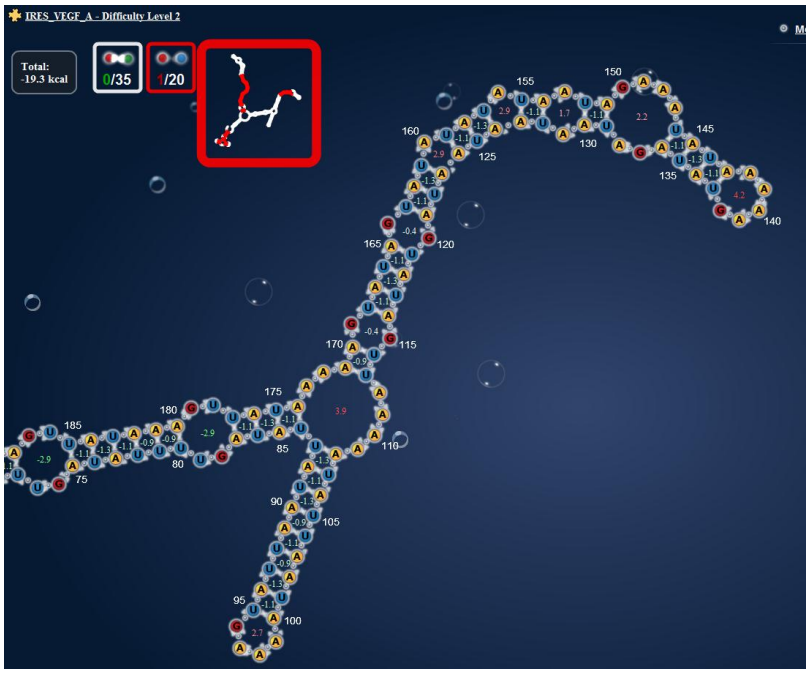
Good



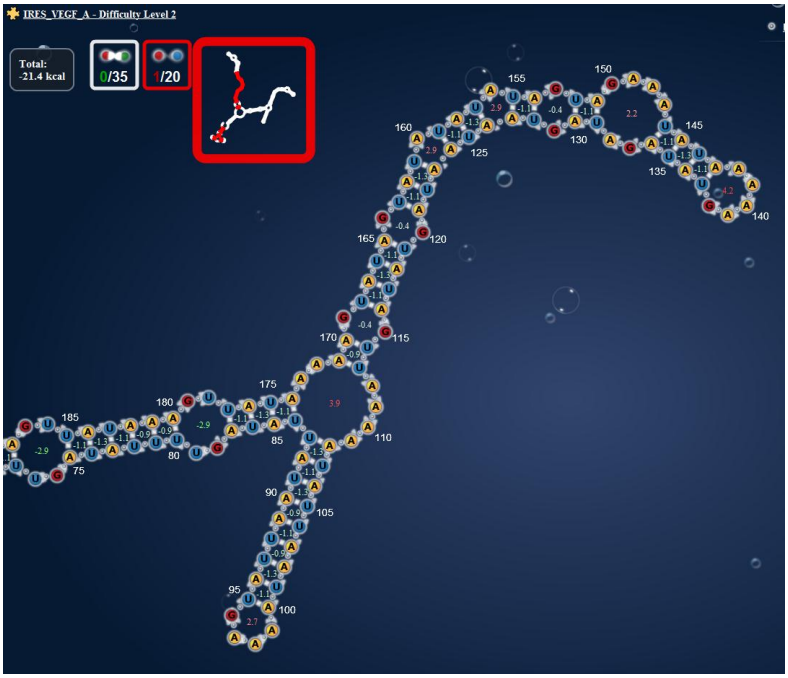
Bad

Figure 27

Now that the unwanted mismatch in the 2-2 loops has been corrected we zoom out again and look at the mini-map to see how the structural integrity has changed. We see that there are 1 major and 2 minor problem areas and a quick look at those problem areas and I realized I had missed a 1-1 loop boost in the minor problem area of leg 2. Observe in figure 28 the change in both the 1-1 loop for the nucleotide used for boosting the 1-1 loop and the resulting structural integrity change.



Before



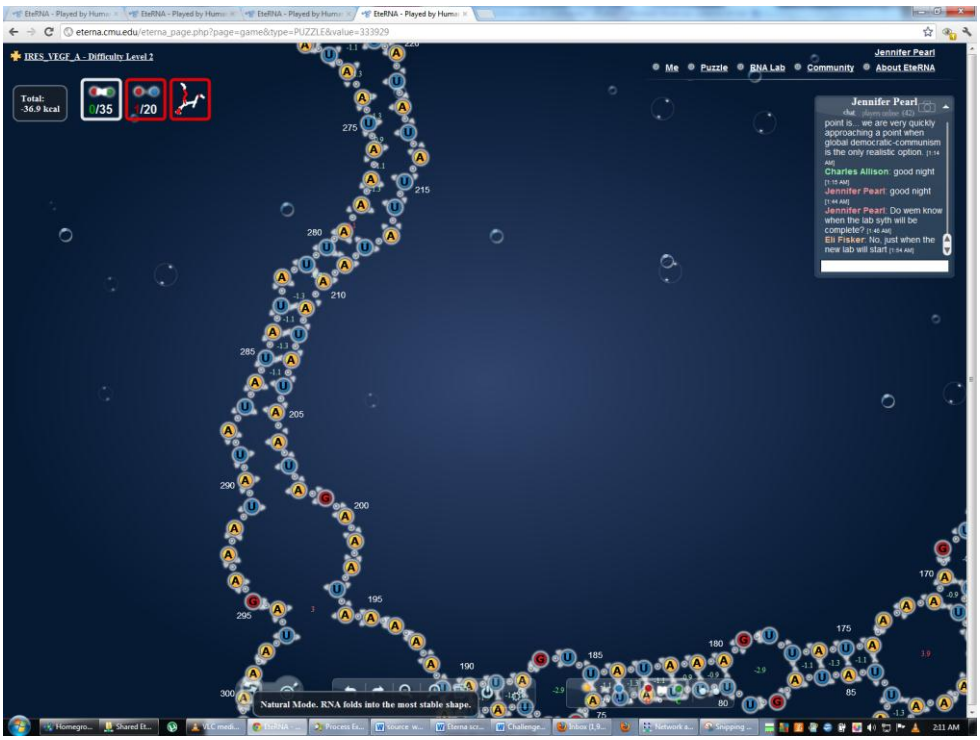
After

Figure 28

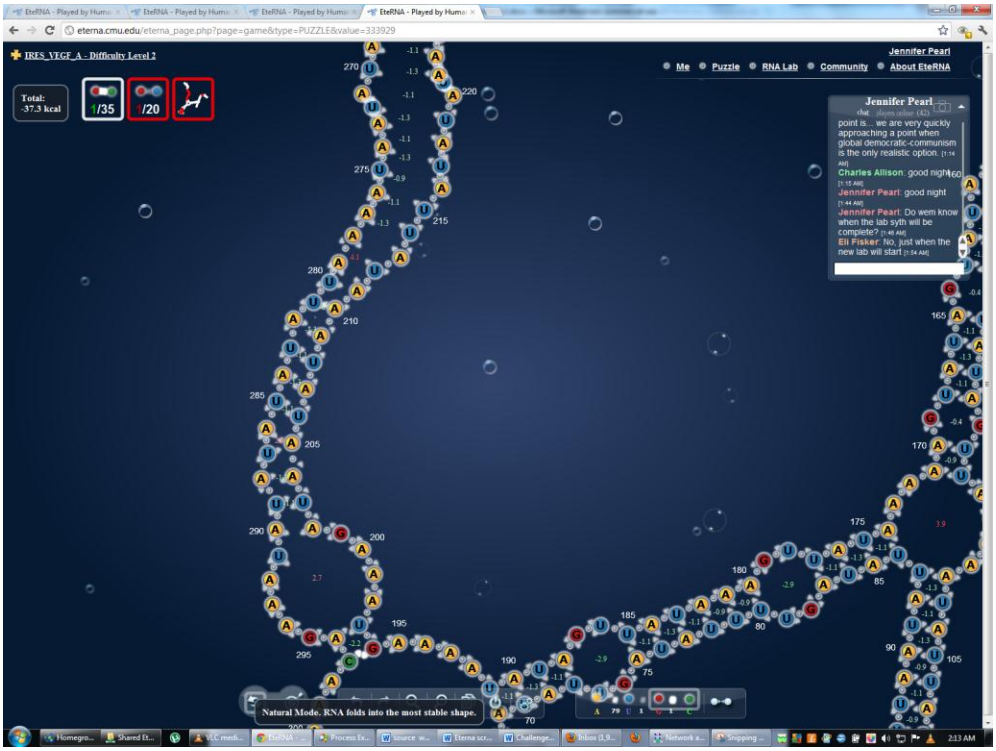
From the “After” section of Figure 28 it can be observed that simple boosting the 1-1 loop properly resulted in a significant improvement correcting the instability created by the 0-1 budge zigzags and achieving complete stability in leg 2. Moving on to the next problem area in severity, which is Leg 1, we see with a close up view that the unwanted mismatch extends from the base loop to half-way up the entire leg. The red stops just at where the first 0-1 bulge is formed if you move from the end loop toward the base loop (Figure 29). Since the instability is located at eth base loop and we know that the RNA behaves as segments of a physical structure so the next course of action would be to secure the segment of the wall to the base loop with a CG pair. Remember for base loops always place the GC pairs with the G on leading edge of the GC if it were to be travelling in a clockwise motion. Observe in Figure 29’s “After” screenshot how the instability closed up a bit and then in Figure 30 the difference between the “Before” and “After” when observing the transition from Target mode to Natural mode. You can see how the tearing is more centralized in the middle of the leg with the 0-1 bulges.

Before After

Figure 29



Before



After

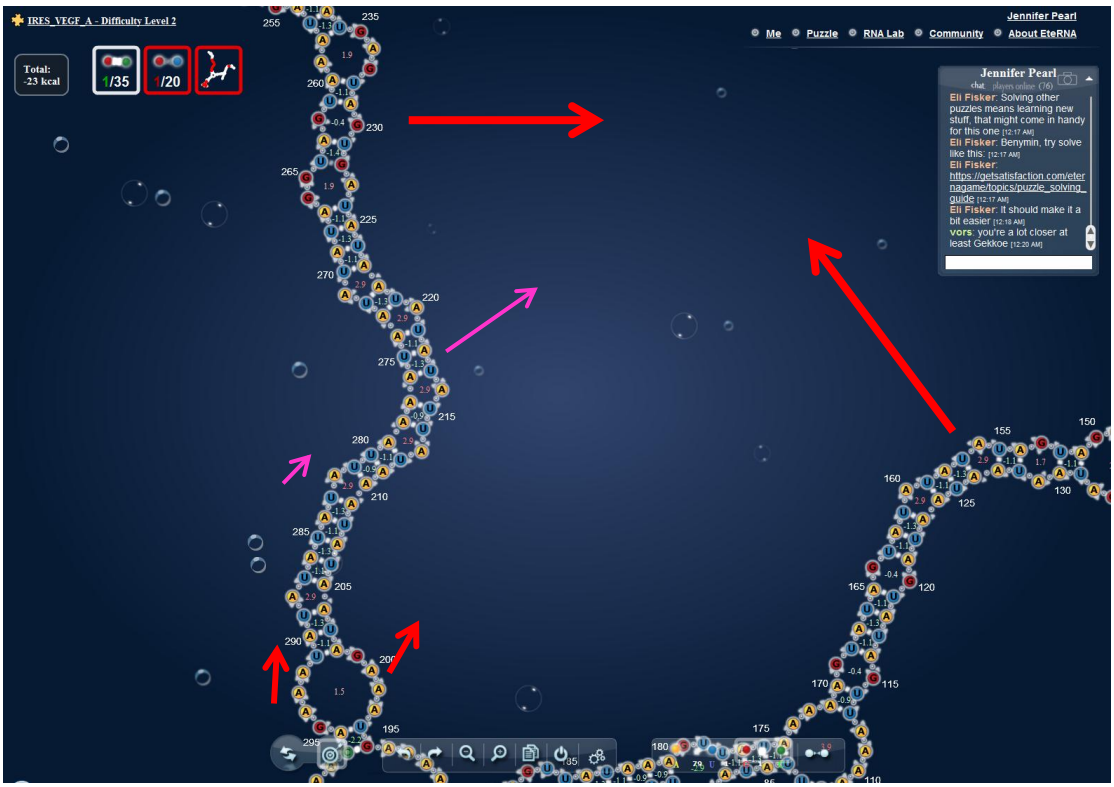
Figure 30

Now that the tearing is centralized we can take on the 0-1 bulges since they still present the most significant problem to stability do to their impact on a main leg segment structure. This is similar to if the wall was to have a support beam ripped out from it, the result would be structural collapse. If someone however was to take a couple blocks off the top nothing would really happen significantly to the structure as a whole. These few blocks on the top would be Leg 3's minor problem area. So addressing the 0-1 bulge zigzags in leg 1 is the highest priority now.

The 0-1 bulge's or zigzags as they are called when they appear in groups must be solved as a group effort treating this section of unwanted mismatch as a segment in itself. The first step in doing this is to zoom in on the trouble area and evaluate the segment under work. Referring to Figure 31 observe the difference between the Target and Natural mode. The red arrows in the Target mode indicate the direction and distance of travel that the area at the point of origin will take once the designed is switched to Natural mode. Think of these as more as vectors than anything else, the longer a line the farther the distance traveled and the shorter the less distance traveled (Note: End point of arrow does not show exact ending point). The pink arrows indicate the 0-1 loops and the red arrows indicate everything else.

We see then that legs 1 and 2 are being pulled together and that once the natural state is achieved the design is still in the same general configuration will very few segments loosing stability. The exception is the area consisting of zigzags in leg 1 which is being pulled into a straight line as can be seen inside the red circle under Natural mode. While the little loop and 0-1 loop near the base loop does shift a little it still retainers its "shape idea". I say "shape idea" because it is not the right nts match but that is because of a design flaw at another point. The "shape idea" however will be structurally sounds enough that it will still retain its shape even if its nts are rearranged (it's a weird concept) which basically means it will be easy to fix later if need be. With this knowledge in hand we can be confident in choosing the section of zigzag and stack starting just after the 1-1 loop moving from the end loop toward the base loop until the end of the boomerang shaped curve. Begin solving for zigzag patterns at a point as close to the end loop for the segment under work.

Locate the first 0-1 bulge and place a single GC pair on the stack on the trailing edge of the 0-1 bulge if moving from the end loop to the base loop with the red G on the lowest numbered nts in the pair. If design stability gets worse then switch the positioning of the GC pair and place the red G on the higher number nts and the green C on the lowest numbered (See Figure 31). If after switching the locations the design stability continues to get worse or does not change from the state before the GC pair was first placed in the 0-1 bulge then return the bulge to the state it was found in and move to the next 0-1 bulge. There is no reason to place a GC pair if it does nothing to the stability of the design at this point. Once an improvement has been made switch between Target and Natural mode gain and observe the change in design stability (Figure 32). We can now see from looking at Figure 23 that leg 1 has increased in stability and only a small portion of the leg needs to be worked on. In fact the natural mode of the design is starting to get more and more like the target mode which is really the goal. Continue making small adjustments following the steps above. At this point you can go through and start placing GC \ CG pairs on the stacks next to any loops that defy any attempt to remain stable. The key to remember is to treat the different parts of the puzzle as segments.

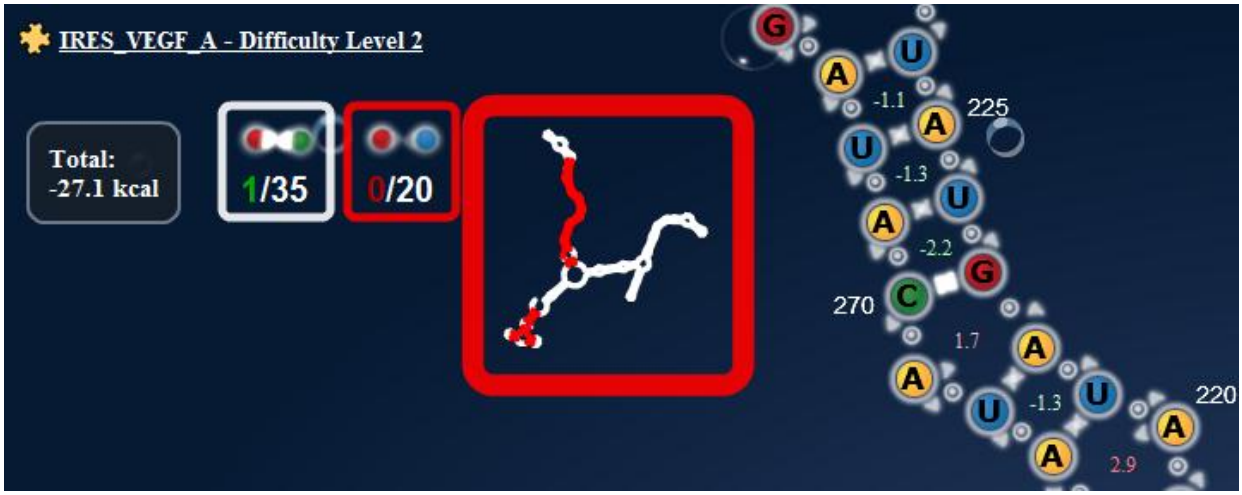


Target

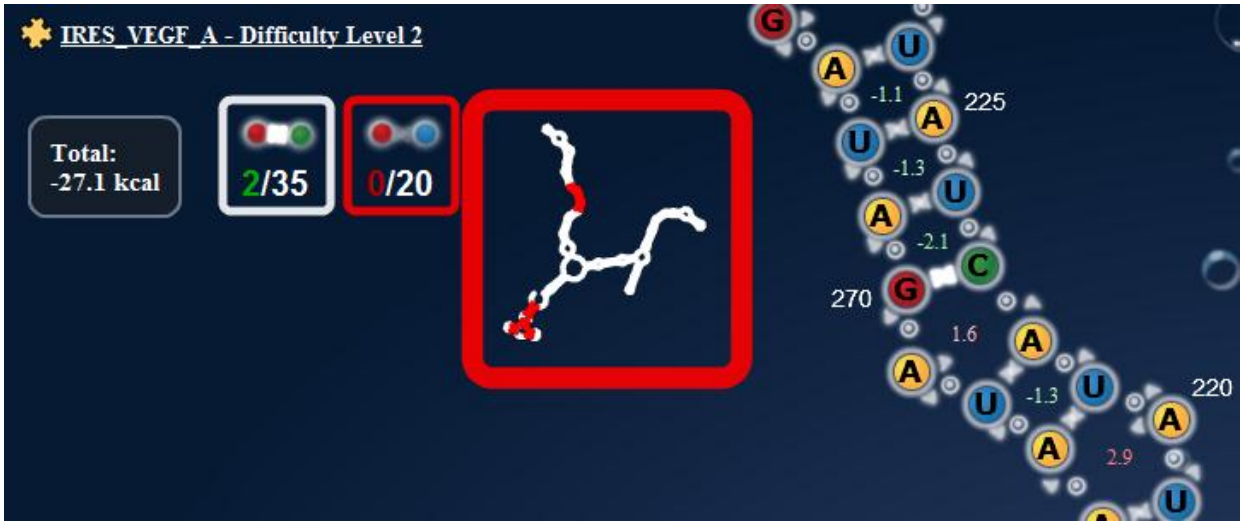
Natural

Figure 31

Before GC placement

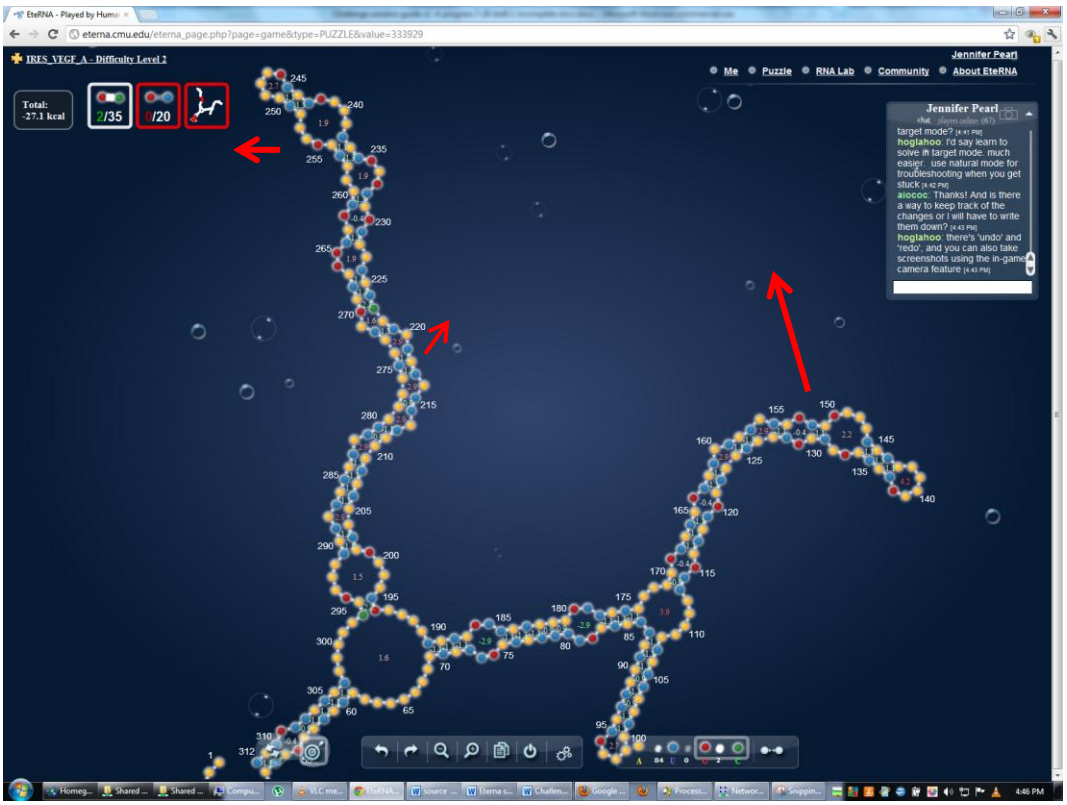


Potential GC placement 1

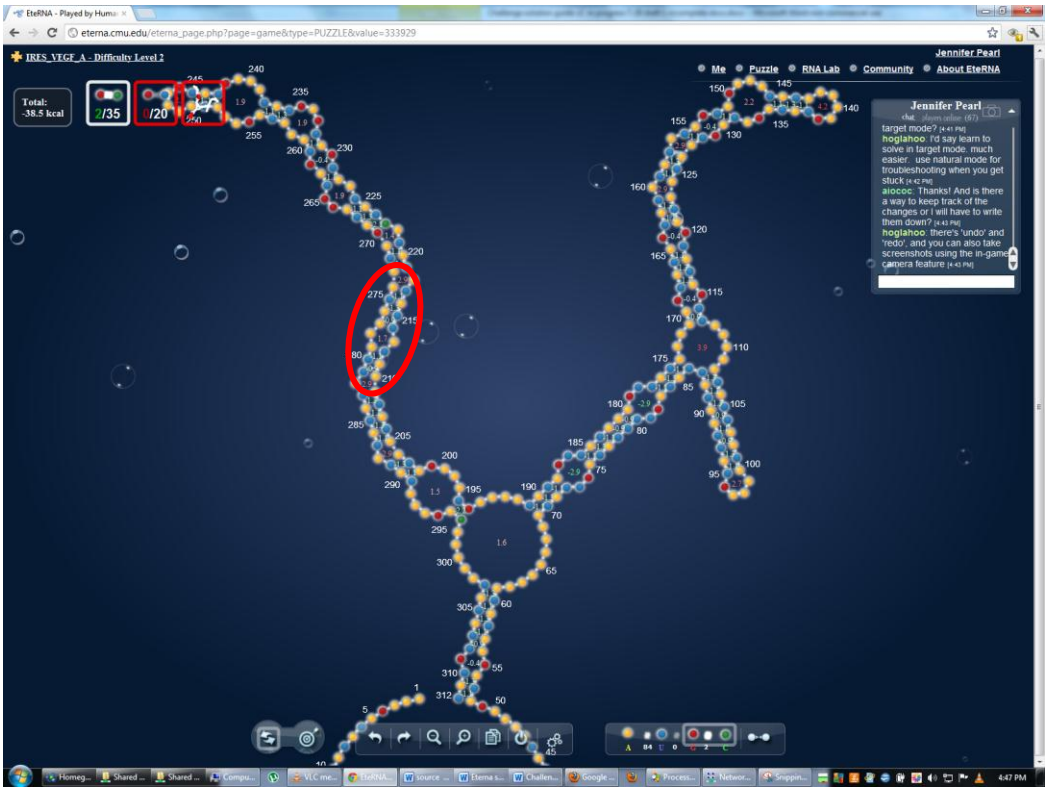


Potential GC placement 2

Figure 32



Target mode



Natural mode

Figure 33

There are many more things that can be done besides what I have spoken about. I hope that this document and the many other documents can help you learn how to solve a puzzle to full structural integrity. When in doubt remember to observe the trouble areas and change AU and GC placements and don't be afraid to play around and experiment.