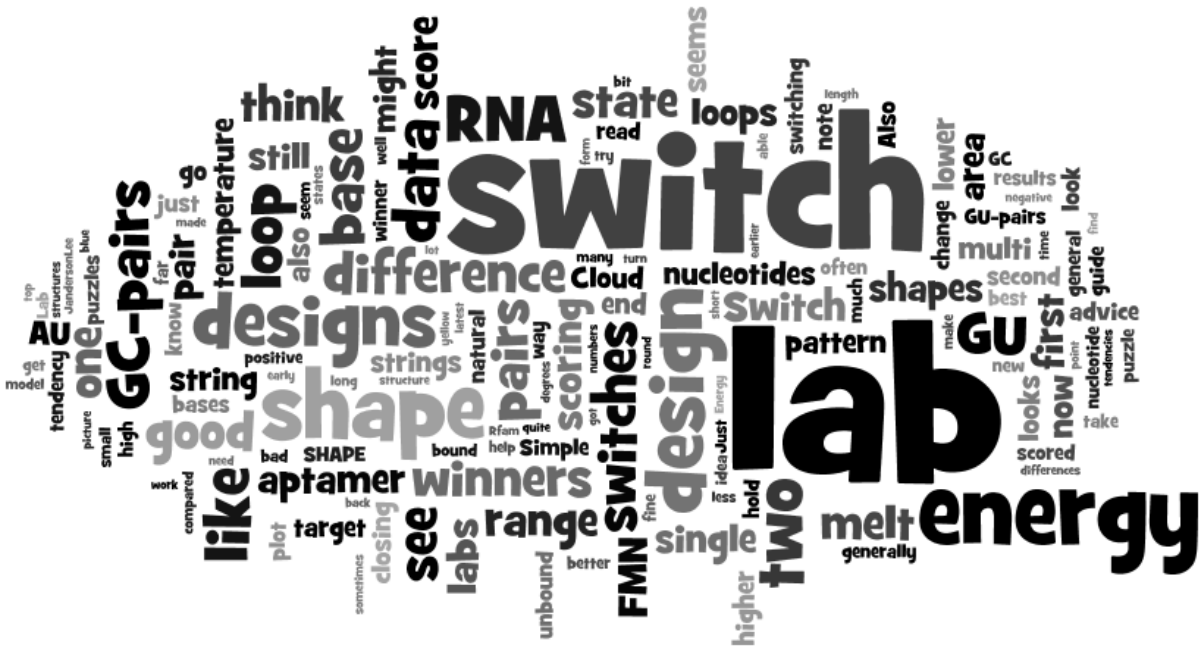
# Switch lab guide




[1]

I have distilled out the lab material from my Switch puzzle guide. If you find the switch labs hard solving, you might want to check that one out first.

Note that the advice given is only pointers. Switches is still very much unknown land. I have tried to share what tendencies I do see and have collected advice for how to go about solving switch labs. So be skeptical.

After we got a nice bunch of switch winners, compared to earlier and a lot of new data, I have added a lot. I made a sum on general Switch lab tendencies. It's a small guide in itself and a superfast way of getting introduced to switches. This guide give an intro to the lab tools and terms, but it is not on MS2 labs. So also check out the newer material on Switch Labs. I also in particular wants to highlight Omei's tutorial which is very helpful for the latest type of switch labs.

I have made this guide with the intention of helping. But remember, the best way to find out things about the lab, is to watch the lab data yourself. Good luck with the lab designing and analyzing!

[1] Thx to Machinelves for introducing me to this visual way to display the content of the guide.

*Eli Fisker*

**New Short Switch Lab Intro's**

Switch Lab Intro
MicroRNA Intro
Doing Math with RNA
Tutorial Outline for A/B Ramp-up Puzzles by Omei

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**General lab info**

Lab tech details and general lab info

These intros to raw lab data are less relevant now as we get our data solely as SHAPE data.

On how to read the raw switch lab data
Visual guide to the new scoring system

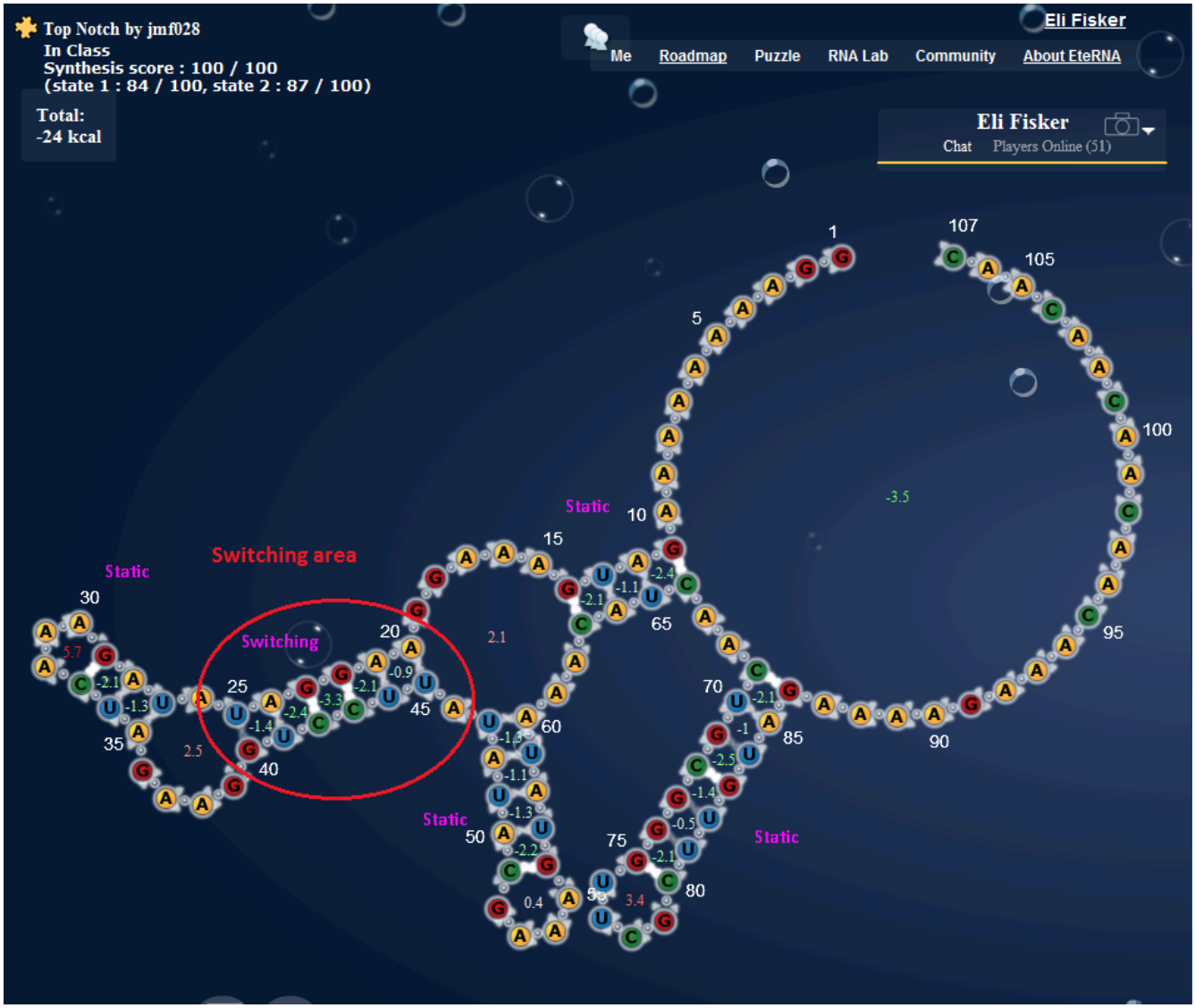
# Switch strategy guide

Now, some time after I started up the switch puzzle guide, I do have some thoughts about what will do good and bad in a switch lab. Note it is still early thoughts and remember I might be wrong about some of it. But I share what I see for now. This section is a sum up about what I have written about switches other places, eg. from my Switch diary.

If this is your first lab, I suggest you later go take a look at my previous lab guide to learn about the lab tools like melt plot and dot plot. It is also a general guide on the process of making a lab design and what to look out for. Or check out Quick Guide to the Lab.

Also remember to do following: Go and watch past winning and losing switch results in the lab results page. It is the best way for you to make your own opinion of what might work and what will not.

## Switching area




In some switch labs the whole of the two states move compare to each other. The area around the aptamer is always the switch hotspot. Both stems around it can be switching or on either side.

## Aptamers

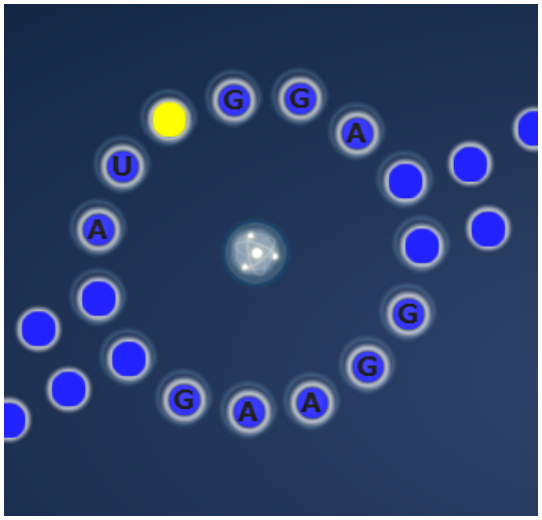
If you have no idea what aptamers are, here is the best place getting started:

Riboswitches and science on coffee

First a short note on aptamers. An aptamer is a loop structure with special nucleotide sequence, that has a favorite molecule it will bind to, if the aptamer folds correct. Here is a  picture of one. Locked nucleotides = aptamer sequence. Notice the molecule inside it.


Tom (one of the developers) described what a binding aptamer would look like. Mat and I have added a picture, that shows what a perfect aptamer should look like in the SHAPE data. For more technical details see here.

FMN aptamer




Normally SHAPE data should be yellow in loops and blue in stems. But as the aptamer is binding to a molecule it will turn blue though it is a loop.

I believe that good aptamers can be recycled just as can good necks. It is still early saying what will for sure work with aptamers.

Reuse perfect aptamers and near perfect from earlier designs. I let the aptamer, so it has two base pairs to both sides with it, from the design it originated in. (Some designs will have locked nucleotides in the area, so this approach won't be able in all switch labs. It also depend on what the two states of the switch looks like.)

I asked Tom if aptamers in both switch and static lab would have same binding pattern and he said yes.. He also gave following further explanation:

Just to let you know, I made sure that all of the cloud lab RNAs were together probed with FMN. The FMN aptamer is still capable of binding FMN even when it is not in the switch. Also, the nucleotides in the aptamer should have the same reactivities in the presence of FMN, regardless of whether or not they are in a "static" shape or as part of a switch. The exception to this is if the switch is not switching all the way!

Another important point to remember is that FMN should only have an effect on RNAs that contain the FMN aptamer. Non-aptamer nucleotides and RNAs without the aptamer should not be affected by the presence of FMN. This fact is important for our new RNA chemical probing strategy, where we probe many more RNAs at once in a single tube!

The last thing means that means that all the RNA's, switch and non-switch can be run at the same time. Which is cool!

**More on aptamers**

- Aptamer position in FMN switches
- Aptamer position in TEP switches
- The aptamer matrix
- Skipping magnet stones on the sequence sea

## MS2 Switches

Since we now have gotten data back from the MS2 lab, I have started to write down what what I think will work.

Technically the MS2 hairpin is not an aptamer, but since it performs a similar function.  In particular because this one shows the strongest pattern of preference of a being placed in a specific spot in a switch design.

- Positioning: Where is my MS2 hairpin?
- Notes on the MS2 lab
- MS2 - Thoughts about the lab results

## GC-pairs

There were surprisingly few GC-pairs in the highest scoring design from the Simple RNA switch. The second highest scoring did have more. I think this is what goes on: This first lab, had only one long string in the unbound shape and two short ones in the molecule-bound shape. A design with more strings, need more GC-pairs. Our latest lab looks like it is happy with round 2 and in some cases (especially neck area) GC-pairs per string.

In FMN aptamer 2.0 the balance shifts to more GC-pairs needed. Probably due to the bulges as mentioned before.

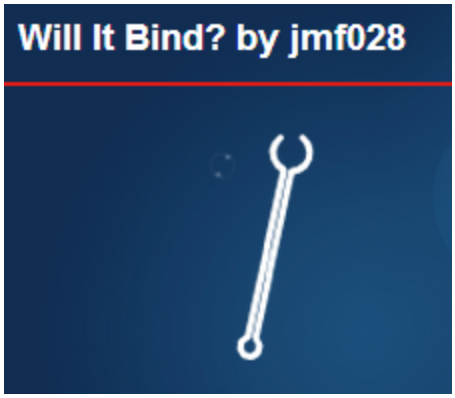
An addition for what I said about GC-pairs for every other base pair. It do look like the neck area have a higher tolerance for an extra GC-pair, this four nucleotide string can often handle 3 instead of two.

So far I think the more strings gets fill onto a switch puzzles, the more needed a certain amount of GC-pairs will be. Though I'm sure there is an upper limit, that will be lower than for single shape lab. I don't think we will see bigger switch puzzles with 80% GC-pairs. I think most will hold a good deal less. I will say that a good switch will mostly have less than 70 % GC-pairs and often it will land in the 50-60% range.

*Cloud lab update:*
Similar to the small lab design Simple RNA switch, winners in the small lab design My screw up, had very few GC-pairs. So this could be a pattern for smaller switches.

I predicted that there would be a lower limit for how high percentage of GC-pairs a switch could take and looks like I was right. It even got lower than 50-60% the range I set out as likely. Now it looks like most of the higher scoring designs land in a 30-50% range. +/- a little The actual winners had a percentage of 23-52% GC-pairs.

The lab, Will it bind? underlines that switches can be solved with really few GC-pairs. The 4 winners had a GC-percentage of 23-30% GC-pairs. Perhaps this will be common for one stemmed long lined switch designs?




Now we only have cloud lab winners 11 winners all in all + 4 from earlier but I think a picture is starting to emerge. Later we will be able to point out more precisely which type of switches will need which amount of GC-pairs. Want to view the data behind this section, check here or go to the lab.

Also see Different types of switches.

### AU-pairs

*Cloud lab update*:
The 11 winners we got so far has an AU ratio between 48-75%. So for now it looks like that as a thumb of rule switches needs a higher AU content than GC content. However the designs with short stems seems to need a higher GC ratio.

In the future when we get more winners we will be able to pinpoint the best AU ratio for designs more precise.

Just know that for now it looks like that small designs like Simple RNA switch and My screw up, will need a higher AU% than other bigger switches. Same might go for long stringed designs like Will it bind?

Simple RNA switch

My screw up

Will it bind?

*Early switch notes*

I think all GU and AU-pairs in a design is a no go. Normally you don't want any string the length of 4 nt's with all AU's no matter how they turn. It usually fails, though Teamshadows got away with it in his Mod top scores (90%) in Simple RNA switch. Longer strings will do fine with a higher AU-pair ratio. An AU ratio around 40-50% will generally do good.

Also see Different types of switches.

## GU-pairs

***Cloud lab update on GU-placement:***
There seem to be a pattern for the switching area itself. If it holds GU's the unbound state can hold more GU's than the bound state.

As JandersonLee nicely summed up:

*Perhaps since GU bonds are not as strong they are more prone to break so that the shape can switch.*

If this the tendency continues, it will be a help, because it means we have an idea about how to make switches switch. Also logically this is making sense.

Below is the description of the process of seeing the pattern.

Only 1 of the 11 switch winners didn't have a GU-base pair in the design. Except for that design without GU-pair, there was a design with 1 GU and the rest of the other winners had 2 or more. It seems as a general rule that Switches like much GU's better, almost to to point of craving them. Many of the top scorers had more than one GU. Still there is a limit for how many GU's can be tolerated. (For now there is a GU-ratio of 0--16% among the winners)

Extra note, some of those GU-pairs are placed in the barcode hairpin and are as such not necessarily needed for the design, but rather an expression for problems with finding a proper barcode without a GU. However Nascarnut's winner TN 3-1 has a GU in the switching area. The same is the case in Hotcreek's winner In class, in same lab.

There is even two GU's in the switching area of the lab My screw up corrected. In Garyfishers design Just another screw-up. The same in Kcalbra28's Albiero 2. And Wateronthemoon's

Bubble Wand? Interestingly enough in the 1 state, for all of them, but no GU's in the second state stem. Is this common? It is the same for Hotcreek's winner in Top notch lab. In Nascarnuts winner in Top Notch lab, two of the GU's are in the switching area in the first state and 1 in the second state.

The long stringed switch Will it bind, the GU's turn up in equal amount in the first and second state. Due to its special structure this one might behave different.

In JMF's winner of the LabPuz02, two GU pairs turn up in the switching area of the first state and 1 in the second state. I find this overall interesting. How did our old switch winners do with respect to this?

In Simple RNA switch lab, the design Another Tebowned mod, did have two GU's in first state and 1 in second state. So does my winner mod of Tebowned.

Neither of the two winners from the FMN aptamer switch 2.0 has any GU-pairs. Might be due to the generally short stems, that they get away with it.

Ok, we still have a rather small sample of winners. I just thought this so interesting that I thought I would mention.

## Lab limits

Nando: long stretches of Gs are known to cause trouble at synthesis stage, hence the limit of max 3 in a row. Also, so far, there doesn't seem to be any stretches of more than 3 UGs in nature (according to PDB), which would make the exercise a quite artificial one (but that's no reason to dismiss it)

*Early Switch lab notes*

GU-pairs seems to to be a bit better tolerated in the Simple RNA switch, than in single shape labs.

For the FMN aptamer 2.0, the picture changed a bit. None of the 3 designs that scored over 90% contains a GU pair. That might also be due to that there are two bulges in the molecule-bound shape and that it takes extra GC-pairs to hold. But it is still quite early to say anything.

Advice for GU-pairs. Max 1 per string, it is a bit dangerous having 2 GU's in one string, at least if it is short. It also seem to be a good idea for now, not putting more than 1 or 2 GU-pairs in pr.

design. Though a few have done ok with 3. I can add now that around 10-11 % GU's are noted in some of the relatively good designs.

Another thing I have noticed is that GU-pairs often do bad when placed at the end of a string. Be it as loop closing base pair or in the end inside the puzzle. However Rfam, database over natural RNA, switches included, disagree. Here Gu's are found at end of strings. So keep an open mind in this matter and ignore my first advice.

I think the longer the string, the more tolerant to GU pairs as closing bases. So in strings longer than 4 nucleotides, GU's at end bases do not fare as bad. Sometimes a GU do close a 4 nucleotide long string and it shows up as stable in the shape data. Just know that the designs with GU's at end bases most frequently show up among the lower scoring designs. For more about it see my robot strategy on it.

[Later comment: Rfam, RNA database which also has RNA switches, shows GU's to do well at end bases, go see for yourself] Here is what I see in Rfam in natural occurring switches:

GU content is relatively low. GU mainly occurs at closing base pair and in short strings too. Both at closing base pair for a loop or closing base pair for a multi loop. The GU-pairs in stem are spread out. Not beside each other. And mainly in longer strings. I did find one double same turning GU at a string end, and a triple one inside a string. So no clear pattern here.

### Closing base pairs

Switches seem to be less picky about end pairs when it comes to strings. Or rather it seems they have a different preference. Even when it comes to GU-pairs, judging from examples on natural occurring RNA switches I have seen in Rfam.

Actually it is a bit opposite to single shape lab, that mostly always needed GC-pairs at the ends. Imagine it like this for the switch labs. If all closing base pairs in strings hold a GC-pair, then the loops will gets filled with loose G's and C's, as this is typically where the bases of a GC-pair when end up, when split from the switch to the other shape. As every color that ends up in loops are open to mispairs elsewhere, having more of G's and C's that has a particular strong pull, is not the best idea. U's and A's have less of a pull. Watch the winning designs and you will see that many of the closing base pairs are often AU's and as seen in Rfam, a GU. That doesn't mean that you shall make every closing base pair AU's. It is sort of a balance. For now it looks like around a 50% of the closing base pairs are not GC's. And that GC-pairs have a habit of being more in middle of a string, as opposed to single shape lab. Watch out for the tendencies in the winners yourself.

To think about: Is there any pattern for where the GC-base pairs turn up?

## Melt plot

*Cloud lab update for melt plot:*

Now we have more winners and with the new lab synthesis method, new patterns arrived too.

The temperature range for the 11 new winners are 77-107 degrees. I foresee winners with a melt temp of 0 degrees. This temperature is due to lab designs submitted that do not satisfy the energy model, and as such can not be estimated melt temperature for. One of these scored 93%. However there still is some tendency for the melt. Most of the winning and high scoring designs stay out of the 107 degree range and are on 97 and a lower.

One thing that will make a switch show a high melt plot and be allowable and fine, is if there are parts of the design not moving and that includes long stems. Long stems notoriously show high melt temp.

*Early Switch Lab Notes*

What I state here is for the unbound shape. The one that gets showed in the lab results. The molecule-bound shape gets a melt temperature too. You can see it when watching dot and melt plot for the molecule-bound shape.

The Simple RNA switch seemed to do good in a temperature range of 67-87.

The FMN aptamer 2.0 so far do good in a temperature range between 77-87.

That still might be a bit too narrow range for both of the labs. But it is nice to know that a melt plot on 47 and 107 is probably a no go.

FMN Switch 2.0 so far do well in the temperature range of 57-67.

For now it looks like the switch labs have a narrower window for temperature range compared to single shape lab. I think this will hold true for the % of GC-pairs a design can tolerate too. Both upper and lower limit.

## Difference in melt plot between the two shapes

***Cloud lab note on melt differences:***

Cloud lab note, the bound shape can have the highest of melt temperatures too, compared to the unbound shape. Eg. check this design. Several designs in the lab “My screw up” follows that pattern.

Melt temp is very different in Cloud lab switches. Zero difference, seems just fine now also the melt temperature is much higher than before. Actually all the winners except for 1 had a melt temperature difference on 0. See data here.

*Early Switch Lab Notes*

I think I have found one more way to help discriminating between good and bad switch designs. It won’t always work, but to know about tendencies for what usually will be good or bad, can be very helpful.

I have been looking at the difference melt plot between the two switching shapes. When I look at the winners, it seems like an melt difference on 20-30 degrees between unbound and bound target is good.

The molecule bound shape has the lowest of the melt temperatures. The best scoring will more often be in that range, though a few good scoring designs can have temperature differences on 40 degrees. Just the lowest scoring more often than the high scoring designs have this big a difference.

My winner in the lab FMN aptamer 2.0 do has a 40 degree difference. But after the new scoring model it will score lower than Drax winner. Which has a 30 degree difference.

Some of the bad scoring designs has a bigger melt difference. A design that scored 23% in the lab “Simple RNA Switch” had a unbound target melt temperature on 87 degrees and a bound target melt temperature on 47 degrees.

And the design Rabbit in the FMN Switch 2.0 had the highest melt temperature at the molecule bound shape instead of the unbound target, unlike the overall tendency. And it was the design that scored lowest in this lab. (old scoring system)

It doesn’t look too good if the two melt temperature are too equal. Like 0 difference, though a few designs do okay with zero difference. And neither if the bound target has a higher melt temp than the unbound target. I’m looking forward to more numbers from the lab.

## Energy difference between the two shapes

*Update: Cloud lab note on energy differences:*

Also here is changes compared to earlier tendencies. The 11 winners were spread on the whole energy difference spectra from 0.23 to 4.6. But there is a tendency for the labs with more winners to lay in the same energy range. So I think we with time will be able to know more about what energy difference is beneficial for a certain type of RNA switch shape.

*Early Switch Lab Notes*

It have been brought up by multiple players back in time. I think it was Wisdave or JandersonLee that latest mentioned that energy difference between the two shapes seemed to matter. Which inspired me to check for myself afterwards. My advice was try keep the energy difference between the two shapes between 1 and 3 kcal, because the designs with lower or higher difference seems to score low. But after the winning design from FMN Aptamer 2.0 only had an energy difference at 0.9, I will say from 0.9 to 3 now. So take note on this when you design yourself or vote for a design. 3 might still be too high. JandersonLee advice a narrower range.

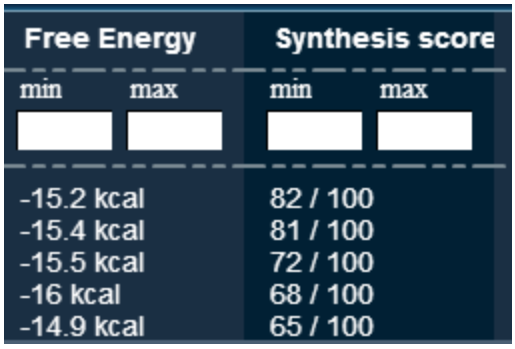
Later addition: The design Starting point scored fine (81%), having an energy difference on 4,6. So it looks like designs are more susceptible to little energy difference between the shape, than a high one. Do however also notice that this design is still not a winner and this huge gap might not be advisable.

And Jnicol's Summers back (92%) were 4.2 kcal apart in energy

## Overall energy

General advice. If there is earlier lab data on a switch shape, watch the general energy range for the top scorers. It gives a hint about what range it is good to be in. Each switch state has a total energy, but our lab interface only records one of them

Think the below example was from our early Simple RNA switch.




For the energy range of the first shape, an energy between about 14-16 kcal looks good. The end range might turn out to be a bit wider. But the best designs is in that range for now.

I sorted the designs after score, by clicking on the top of the column. Then you can see which energy range the best designs are in. This will can give you an idea about what is a good energy level for that lab, if it is a second or later round. At least for single shape labs, it helps checking past designs of a similar shape, containing similar structure and with similar length stems. It will give you a pretty good idea about what the design you are trying to solve, will tolerate best.

## Stem length

Another picture that is beginning to paint itself is also that stringlength have something to say for the distribution of base pairs. Like in single shape lab, designs with lots of short strings, in the switch lab FMN aptamer 2.0, was much more tolerant/ craving GC-pairs, to get hold together. While the Simple RNA switch that was 7 nt long in the unbound shape, could live with much less GC-pairs. Even in the molecule-bound shape. Which is something I don't quite understand yet. I think it must be because there were two small strings adding up to the binding strength together, but I still find it odd that so few GC-pairs could hold the structure together.

## 2-2 loop

### FMN Switch 2.0

So far I detect a small tendency for the top scoring designs having a rather positive 2-2 loop. I'm basing it on thin material. 3 top scores have it. And I detect a slightly more negative tendency for the lower scoring designs.

I have put the numbers for round 1 and 2 in this spreadsheet.

I have later added numbers for round 3 and 4 too, that scored above 68% correct folds. It looks like energy between 0 and positive to 2 point something will do good. Especially the slightly positive seem to do well. The 2-2 loops with negative energy more often ends up in lower scoring designs.

### Energy in internal loops

I still think energy differences play a huge role - that is differences in energy between the two shapes. I think this is why the loops usually don't work if having too negative energy, as I think this will stabilize the first shape over the other. Also loops don't work if being too positive, if you take the winners and look at their energy differences.

### Multi loops

*Cloud lab note:*
This tendency for multi loops being more positive than in static labs, continues.

Multi loops in switches can very well be solved with 2 AU and only one GC as closing base pairs.

For latest addition on multiloops in switches, see here and here.

Later addition (For FMN Switch 2.0:
A picture for good multi loops in switches are starting to get clearer. As I suspected, having too high positive energy there, was not a good idea. The 5 designs scoring 65 and above, have an energy in the multi loop on 0.4-0,6. To get that, one generally needs two GC-closing pairs in the multi loop. But having the energy as low and negative as is more typical in single shape lab, don't look to be a good idea so far either. Neither do very positive numbers.Time will tell on that one. For now it looks like it should be kept in a slightly positive range. For the numbers themselves, see this spreadsheet. This tendency 0.4-0,6 continues strongly after I added in round 3 and 4 numbers in the spreadsheet.

Also a look in Rfam, database over natural RNA reveals both AU's and GU's as closing base pairs for multi loops too. Generally multi loops still has a number of GC-pair closings, but it seems to be necessary not to have all of them be GC-pairs.

Having too many GC's following orientation as good for many static lab multiloops, can be counterproductive. Making the multiloop too stable to want to switch.

*Early Switch Lab Notes*

Speculation: I strongly suspect that GC-pairs in multi loop still are quite important. Not as closing base pairs for all strings. But I suspect that 2 out of 3 gc-pairs in this lab, needs to be GC-pairs and that direction still matters. Namely in the same direction as for the single shape lab. That is the tendency for the designs made so far and what the energy model prefers for the puzzle solve. I'm looking very much forward to see the lab results.

## Repetition

It actually looks like repeats are better tolerated in switch lab than in single shape lab.Two GC-base pairs turning sameway will often go fine, same with a double AU-pair. The repeats also occur more on strand basis. There are more repeats than in the single shape lab.

### Switch lab patterns

Another thing I have noticed for a while is that GC-pairs generally likes to have another pair between them, like an AU-pair, before the next GC-pair. Not a 100 % rule, as two GC-pairs do go fine beside each other sometimes too. Both turning same way and switched opposite.

### Closing base pairs at neck/hook

As for the closing base pair at neck/hook end, switch labs (for both shapes) seems to have a preference for less negative energy than the single shape labs, which most often prefers a hook energy on -1.9.

### Different types of switches

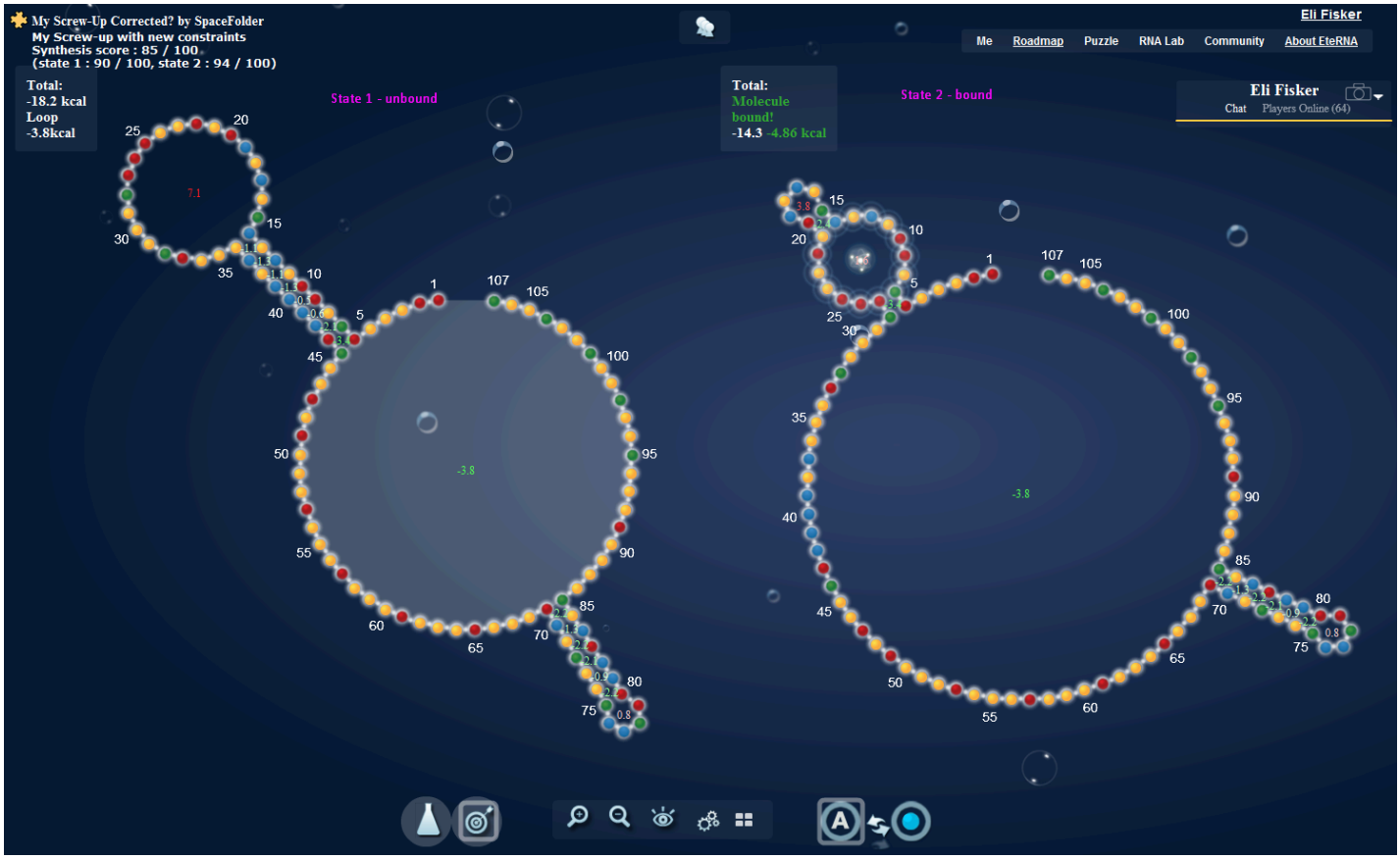
There seem to be different types of switches. Switches that only move a little part of the two shapes compared to each other, some that moves partially compared to each other and then some that does a full move. Actually there seem to be a pattern in direction of that the full moving ones, generally seems in general to take a higher AU amount than GC. Also that the partially moving ones seems in general to take a higher GC amount than AU. (LabPuz02 seems to be an exceptions on the latter)

It is quite logical that those which change fully, has a tendency to want to have more than AU than GC, as the AU is weaker than GC, and therefore is easier to break, which is necessary when all the base stations need to move spot relative to each other. Yet it is quite nice to know in advance that because this design change completely, so it must have fewer than AU GC. The only partially changing generally want more GC pairs than AU.

You can read more about it here and here.

## Tips for watching the lab data

I have recently begun watching switch lab data results in a different way that I find useful to tell me how well the switch has happened and if the lab design is working.



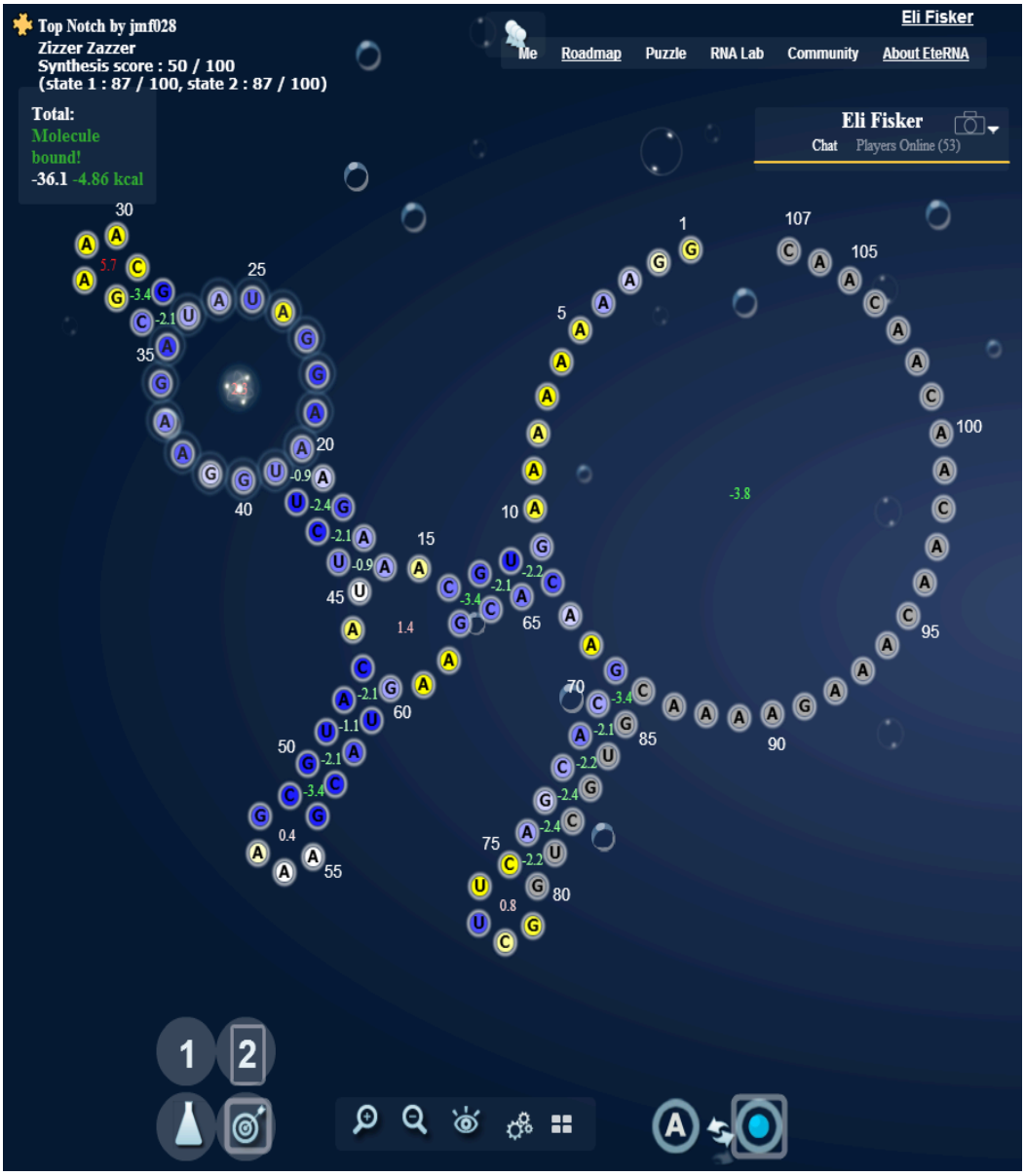

When change from Target shape to Estimate mode, like below, I can see how both shapes are estimated to fold up.


You can do the exact same for with the SHAPE data too.


And then switch from target to estimate, to see how both states are estimated to fold up.

## How to read the collective switch score

By the way, I think I understand the scoring system a bit better now. The design Zizzer Zazzer made me wonder about its collective score, but also helped me see why it scored the way it did. It scores 87 in each state, but just 50 when looking at the collective score. Looking at the second state it is almost perfect. It breaks apart where the yellow bases are are in the stems.




But what will explain why it is not scoring higher than 50, is if you look at the estimate of how first and second state folds. They are very close, meaning that the first state doesn't form at all. So the switch is not occurring and thus the low score.


So the collective score isn't just an average of the scores for the two states. But also a score for how well the design switches between the two states.

In another of the outliers, JMF Top notch with an multi loop energy on 2.4 in the first state multi loop, there isn't either any switch. The design seems frozen somewhere partially towards the second state. I found it particularly interesting that these outliers showed a clear preference for the second state, sort of indicating that to have the second state form, energy in first state multi loops that are connected with the switching area, must not have too much negative energy.

# Advice after the first switch cloud lab results

This was the original start on this guide, with the advice I gave when the switch labs were new. Some of it will still hold, and I have added in updates on these tendencies that changed direction and those that turned out not to hold.

## Loose nucleotides in hook and ring loop area

Green have a strong pulling power so my first advice will be, don't place a lot of loose C's anywhere you don't need them, that is not intended to be a string. So don’t place them where they are single in both shapes, like in some of the loop ring. They even makes trouble if they end in string in bound target, but not in unbound target.

[*Cloud lab note*: One green have shown to sometimes be necessary, if it works, leave it there.]

What I think will be general for the switch labs is following: Don't place nucleotides at places that won't end up in string area, unless you have a special purpose with doing so. Especially if those nucleotides are green or red. Even two blue beside each other in a loop ring can make trouble. But since it is unavoidable, do allow some.

[*Cloud lab note*: Other colored nucleotides have turned out to be necessary in the loop area. Just be careful with how you place it. Don’t place 3 of the same colored nucleotide in line (except for blue in some cases) as they might pair up unwanted elsewhere. Be careful with placing G and C too close as they both have a strong pull and will happily help make a switch. So don’t put them too close outside of the actual switching are, but do exploit this fact for the switching area itself.]

And double and triple and more nucleotides of the same color (other than yellow) generally seems to be a problem in loop ring too.

[*Cloud lab note*: Other colored nucleotides outside of string area have proven to sometimes be necessary, just as it lately turned out to be the case in static labs]. It was recently discovered that more than 4 yellow in line caused problems, that this might explain why there was so many single red nucleotides in the hook ring of the first switch lab winners. JandersonLee says that 5 yellow nucleotides in a row seems to go just fine. The SHAPE confirms this, so this is what I will recommend too.

## Making a mod of a switch lab design

Advice on modifying an already scored lab design. If you are doing a mod of a design that scored good in lab, but can still be improved for the next round here is what you should know:

If you try to fix too much, you might end up fixing nothing at all. It is my experience from earlier labs, that it often ends bad, if I try too much at the same time. Switches are a lot more touchy than static labs in general. Just even a single nucleotide change can cause a dramatic change in the design score. Like a score change of around 20. (As a single nucleotide can easily affect how well the RNA switch does switch. When you change a lot and it does not goes well, then it will also be hard seeing which of your fixes made things go worse. By changing a little you may better learn from what happens, be it good or bad.

Also you shall remember that with the switch lab, you are not just changing one base pair or adding a nucleotide, like in labs with a single target shape. Here you are potentially changing one nucleotide + how it reacts to it's partner and 4 neighbouring nucleotides in both shapes. So go for a nice improvement instead of losing points trying to fix everything. Update, this have proved to be particularly true for switchlab. Even one nucleotide can have a drastic change for better or worse score.

## Understanding switch designs better

Hoglahoo had some really interesting thoughts on the switch lab in comparison with regular puzzles:

...just thought of something else that is sort of an analogy with the switch puzzles. When working the switch, the hardest thing for me was switching between the target shapes and treating them as separate puzzles, yet remembering they are related and some bases need to not change. In regular puzzles where there are misfolds, the same idea exists between target and natural mode, because the natural mode is a puzzle in its own right. The difference is that unlike the switch where you try to encourage both, you try to discourage the natural.

So not to just take a passing glance at the natural and try to get away from it back to target where it's' comfortable, it helps in some puzzles to really look at the natural for anything that you know might help destabilize it if it was in fact the puzzle you were trying to solve, not just blocking a pair, but sometimes using little other things that raise the energy of a loop or multi loop. (From chatlog 8-6-2012)

### Advice after the first switch Cloud lab

I have started adding in what I think I see after we received back the cloudswitch labs data. Just be aware that our data sometimes gets changed, if flaws are discovered. So what I mention here, might not hold. I will change my advice, should our data change.

There is many double C's (dinucleotides - two bases in line in a strand) in the high scorers compared to the lower scoring) Hmm, which lab? I think this advice ended out of its context.)

### Energy difference between the two switch shapes

It have been brought up by multiple players back in time. I think it was Wisdave or JandersonLee that latest mentioned that energy difference between the two shapes seemed to matter. After discussing it in chat and I checked myself afterwards. My advice is try to keep the energy difference between the two shapes between 0.9 and 3.6 because the designs with lower or higher difference seems to score low. (I have later seen winners in the range of an almost winning design with a energy difference from about 0.6 - 4.6) So take note of this when you design yourself or vote for a design. JandersonLee is advising a narrower range than me and he could be right.

### Blue and green pattern

If you read about that blue/green pattern that I found in many of the earliest switch puzzle and lab too, avoid using that pattern in lab. It turned out that pattern was a byproduct of the experimental setting, created by the way the locked nucleotides was placed in the switch puzzle. This pattern won't work for the latest batch of switch puzzles or in lab either. The natural switches do not contain this pattern. For more about this read here.

On the other hand it do seems like switches really like periodic repeats, where single shape lab generally hated any kind of repeats like repeat base pairs. They were best tolerated in longer strings.

If you want some early thoughts on the switch lab and switch puzzles, see my Switch diary. Note that it is very early thoughts, so be skeptical as some of it is probably wrong. The newest stuff is at the bottom.

### Theophylline Ribozyme Switch lab

There has been a lot of discussions and talks about the new Theophylline Ribozyme Switch lab. We are still trying to figure out how to best take a go on it. I have collected some of the official material on this lab in a doc together with chatlog discussions, to help aid our efforts. EINando888 has been making a very fine intro to the lab and on how to view RNA in 3D.

Discussion on the Theophylline Ribozyme Switch
Cleavage suppression score

*Cleavage score*
It looks like the two scores has some kind of relation to each other so the score will be about the cleavage without Theophylline divided with the score for with theophylline.

Jee explains: A note : the cleavage score that show up in the game are rounded, so it may seem like dividing those 2 numbers do not exactly match the suppression score.

# Answers to switch lab questions

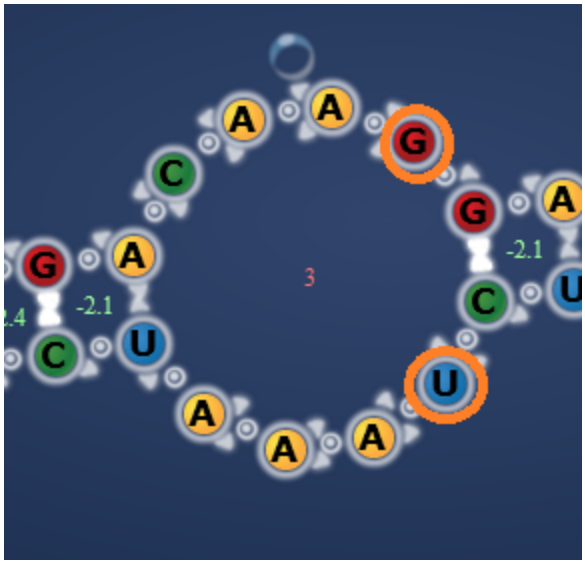
## Will “base pairs” in loop region form a stem?

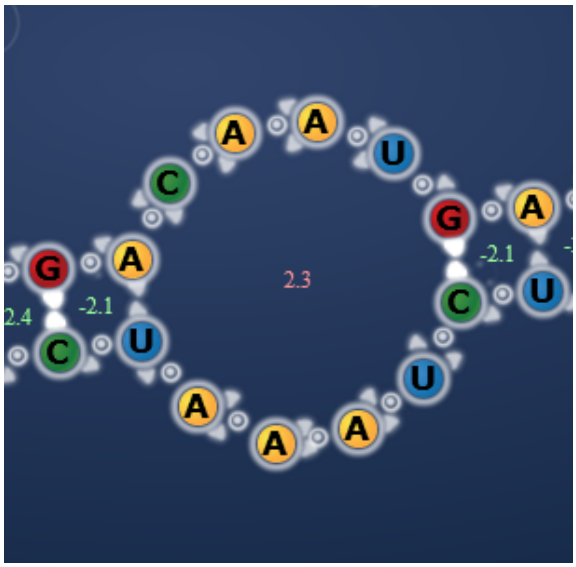
Several players have expressed concern that bases that normally will form base pairs, are opposite each other in the loop openings.

Here are a couple of examples, taken from lab puzzles:

Tetraloop


Internal loop



In the last one you can see that the energy inside the loop got better after the change.

I asked Jee to be sure I understood it correctly and he gave following fine explanation why the above is no problem at all:

As far as my knowledge goes, the model does not consider those as "base pairs."

For example, in the tetraloop situation, if U&A paired up it would create a hairpin loop of length 2, which is impossible in the current model (length 2 hairpin loop has very high energy). Thus, the model decides that those U&A does not pair up and do not consider them as a base pair.

The same goes for the G-U and U-U in the internal loop. In the current energy model, G-U pairs does not always lower the energy - they sometimes makes the energy higher than when they were not present (This is why there can't be a stack full of GUs). So in the internal loop case, the RNA decided not to pair up G-U for the sake of lower energy. The RNA pairs up bases because it can achieve lower energy. If a base pair increases the energy, the RNA will decide not to make the pair.

### Is natural RNA dark blue in SHAPE data?

Eli: I was reading about T-even Phages and one of them having a rather unstable RNA product, and a thought hit me.[2] Is natural RNA in general superstable, when viewed as SHAPE data? (deep dark blue for strings, clear yellow for loops).

Rhiju: Actually, its not clear. I wanted to point out that *random* RNAs can give SHAPE data that have high contrast too -- this is something my lab has recently discovered and is now writing up. In those cases, we have evidence that the RNA does form stable structure, but in most cases it forms an ensemble of multiple stable structures.

What that means is that if you see experimental SHAPE data with 'dark blues and clear yellows', you won't necessarily be able to infer the stable structure underlying the data -- or the set of multiple structures.

That's why in eterna we prefer for you to come up with hypotheses, and then use the data to falsify or help validate your hypotheses. This is different from looking at a virus, where there are potentially thousands or millions of structures that agree with the data, and if you don't have a specific prediction to test you can fool yourself into thinking you know the structure.

[2] Virology - Molecular biology and pathogenesis, 2010

I got so happy about this answer that I just couldn't help it. This poem happened:

*SHAPEDANCE*

*Ribonucleicacid,*
*dancer in the dark*

*One shape of yours*
*cast shadows*
*of thousand stable states*

*Shapeshifter,*
*limited by time*
*you are thousand + 1*

*But which?*

(Feb 2013)

Cody later added this funny note and some beautiful art illustrations. Hereby it is passed on:

codygeary: @eli Shape score is like looking at a shadow and trying to figure out what is casting that shadow. (11 Dec, 2014)

http://beautifuldecay.com/2010/03/10/shadow-art/

## How accurate is the estimate mode?

Eli: I have been wondering about how accurate the estimate mode shows what goes on in a misfolded RNA. I am thinking about the accuracy, because I am wondering if I will be able to use patterns I see in the misfolds for anything. Mat made me realize sometime back that we might be able to get some extra data from the misfolds.

Rhiju: It may not be very accurate at all. We now know several cases where it gives the wrong answer -- see, e.g.:

http://www.stanford.edu/~rhiju/ErrorsSHAPEstructureBiochem_KladwangVanLangCorderoDas_ALL.pdf

The other thing worth noting is that an RNA with the 'wrong' SHAPE pattern may actually be interconverting between several structures.

Our lab has developed a way to infer these structures, but it requires carrying out a more detailed experimental technique called the mutate-and-map method. We are actually carrying this out on several eterna player and NUPACK solutions over the next month or so, and will post in the game... (March 2013)

*Written Dec 2012, last updated Feb 2016*