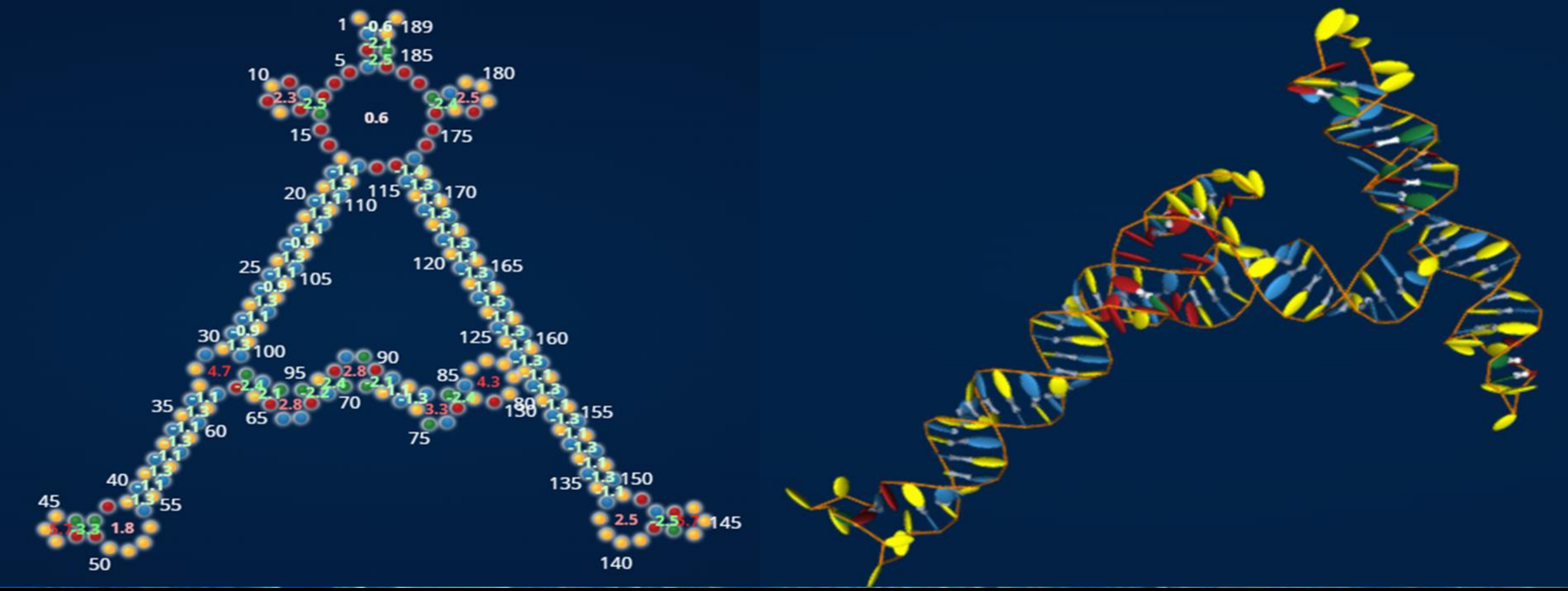
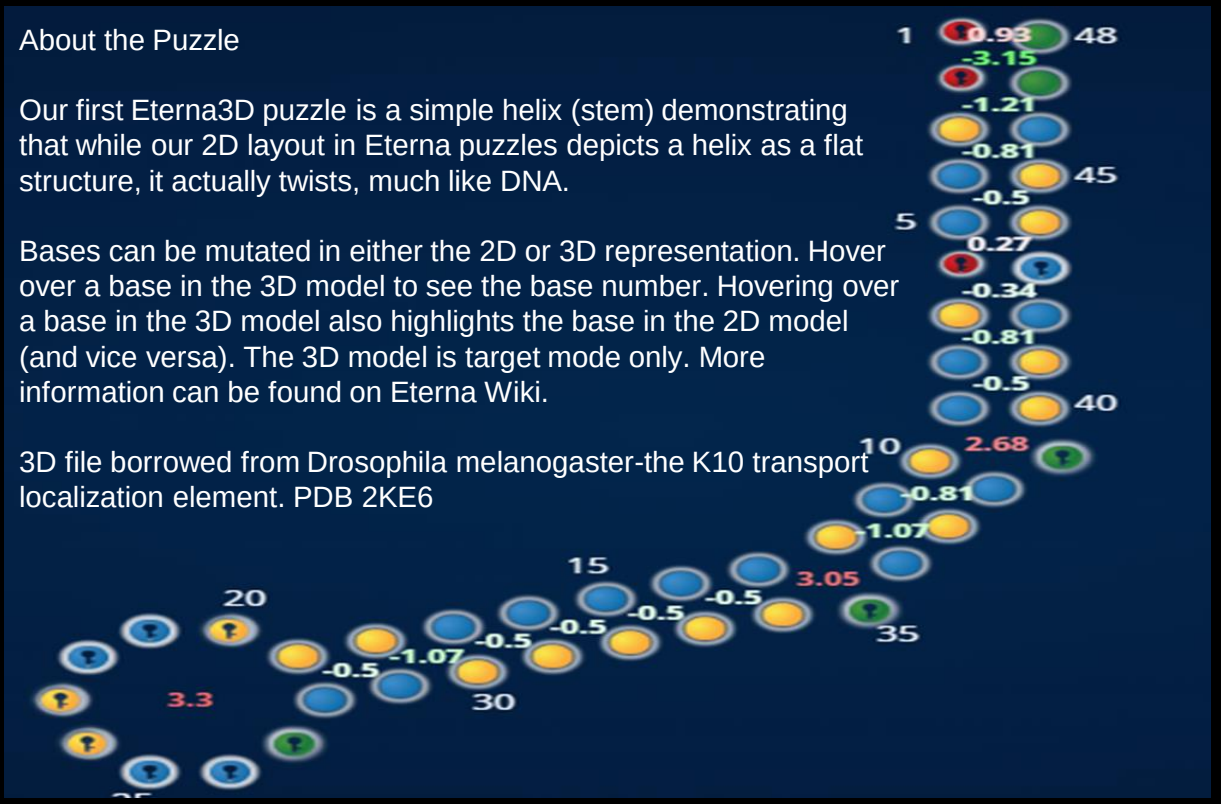
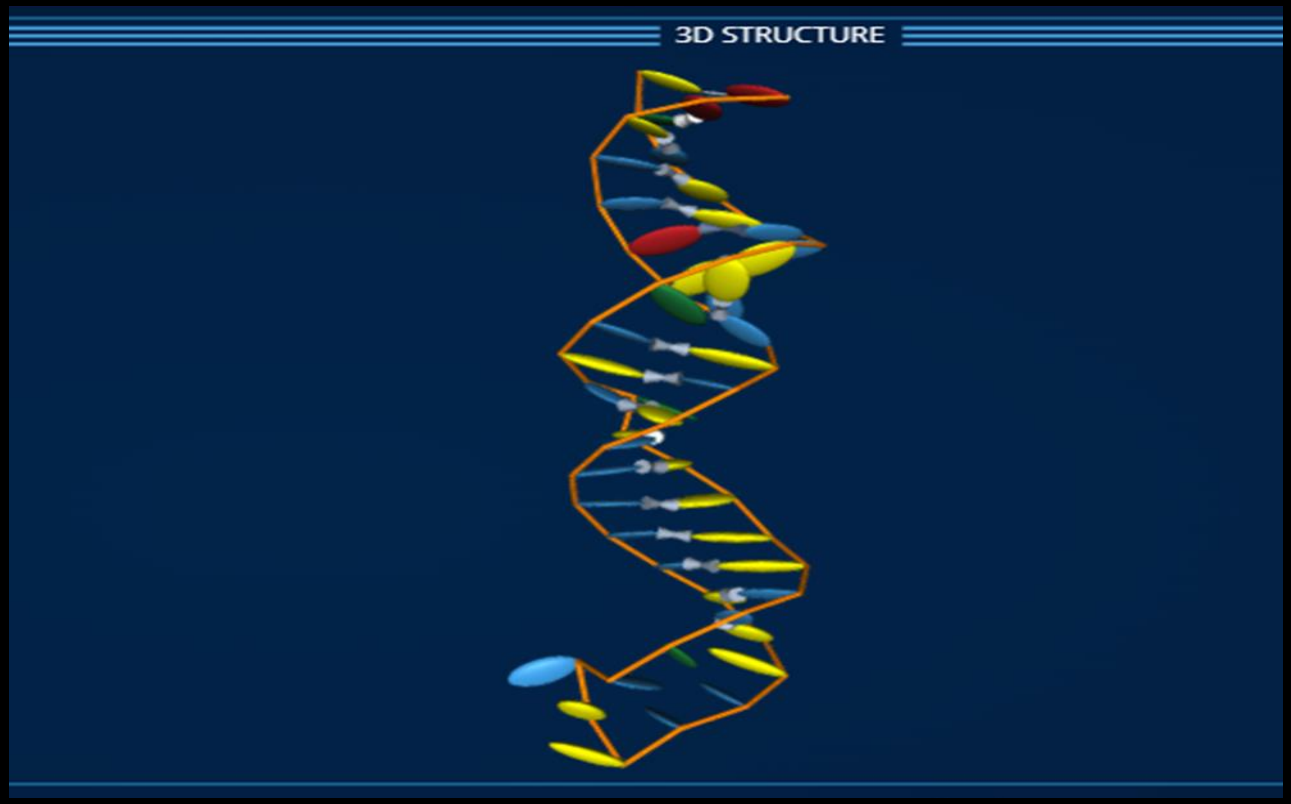


“Building Your Own 3D Puzzle”

by Craig Lollin [UserName: clollin] ~ 3/21/2022

This looks pretty cool...How can I create a 3D Eterna puzzle?





The First Eterna3D Puzzle - https://eternagame.org/puzzles/11213497

What are the steps to creating a 3D Eterna puzzle?

Overview

DigitalEmbrace

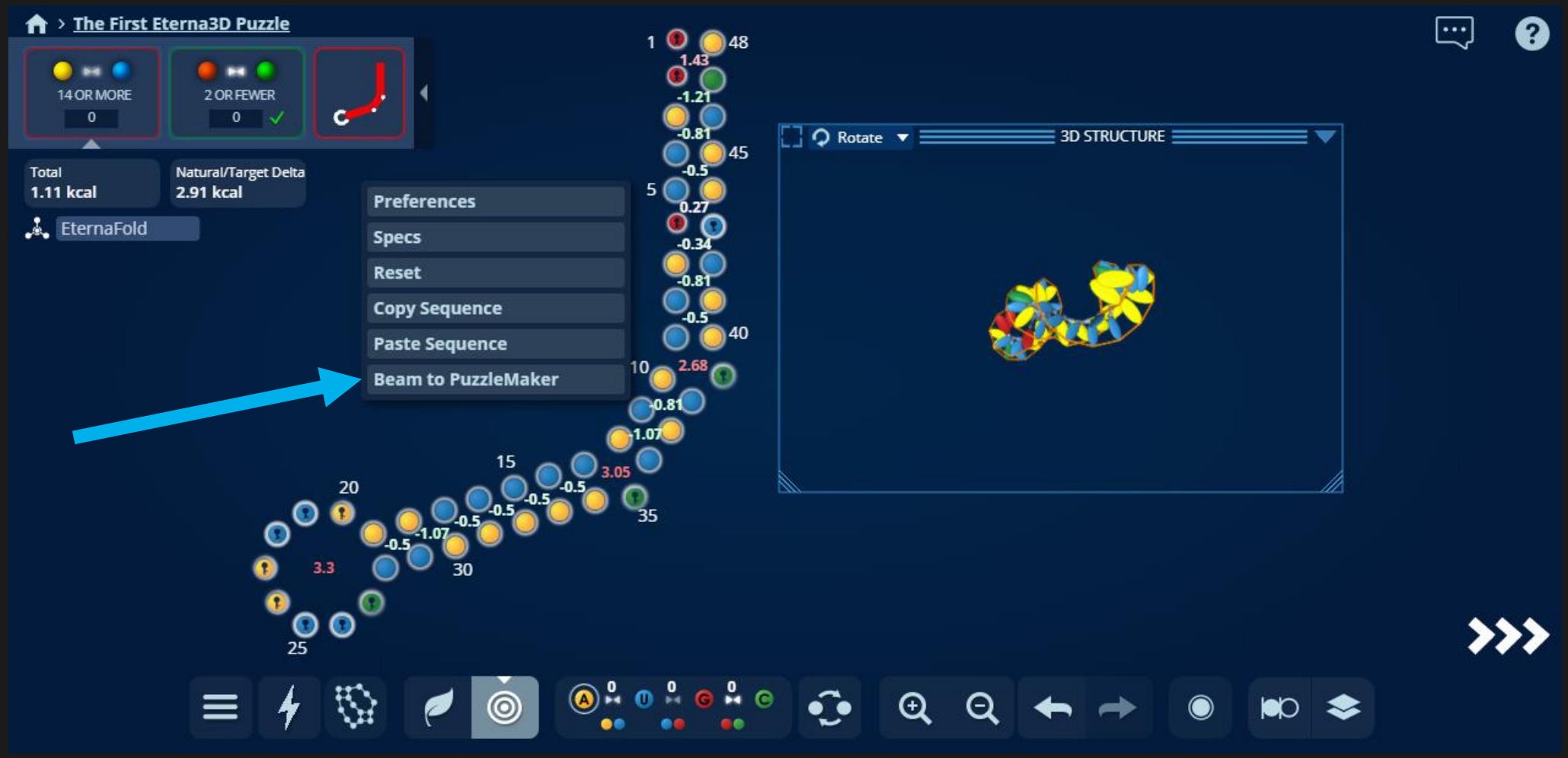
15 Feb 2022

@clollin Are you going to be the first player to create an Eterna3D player puzzle?

- My intro to this was: "**The First Eterna3D Puzzle" -** https://eternagame.org/puzzles/11213497
- **I suggest you go there and read "About the Puzzle" as well as the "Discussion" section for a thorough background.**
- **Summary – I solved the puzzle and posted** ***"I like being able to see that 3D interface! Not that this is a challenging puzzle, but with the PDB accession number listed, the FASTA file and sequence is easily accessible to solve (or at least get close)."***
- **DigitalEmbrace provided a sort of challenge to me –** ***see above...***
- ***Well...I can't let a good challenge go unaddressed, so I was off to figure it out.***

Research – How did they do this? I want to be able to do this! How am I going to do this?

- Beam to PuzzleMaker – what did they do?



Research – How did they do this? I want to be able to do this! How am I going to do this?

- Beam to PuzzleMaker – “Load 3D Model”
- “WHAT 3D Model are they loading???” – basically asking for a file

Research – How did they do this? I want to be able to do this! How am I going to do this?

- Beam to PuzzleMaker – what did they do?
- “WHAT 3D Model are they loading???” –
- “3D file borrowed from Drosophila melanogaster - the K10 transport localization element. PDB 2KE6”

PDB is the Protein Data Bank - https://www.rcsb.org/

RCSB PDB (Research Collaboratory for Structural Bioinformatics PDB) operates the US data center for the global PDB archive, and makes PDB data available at no charge to all data consumers without limitations on usage

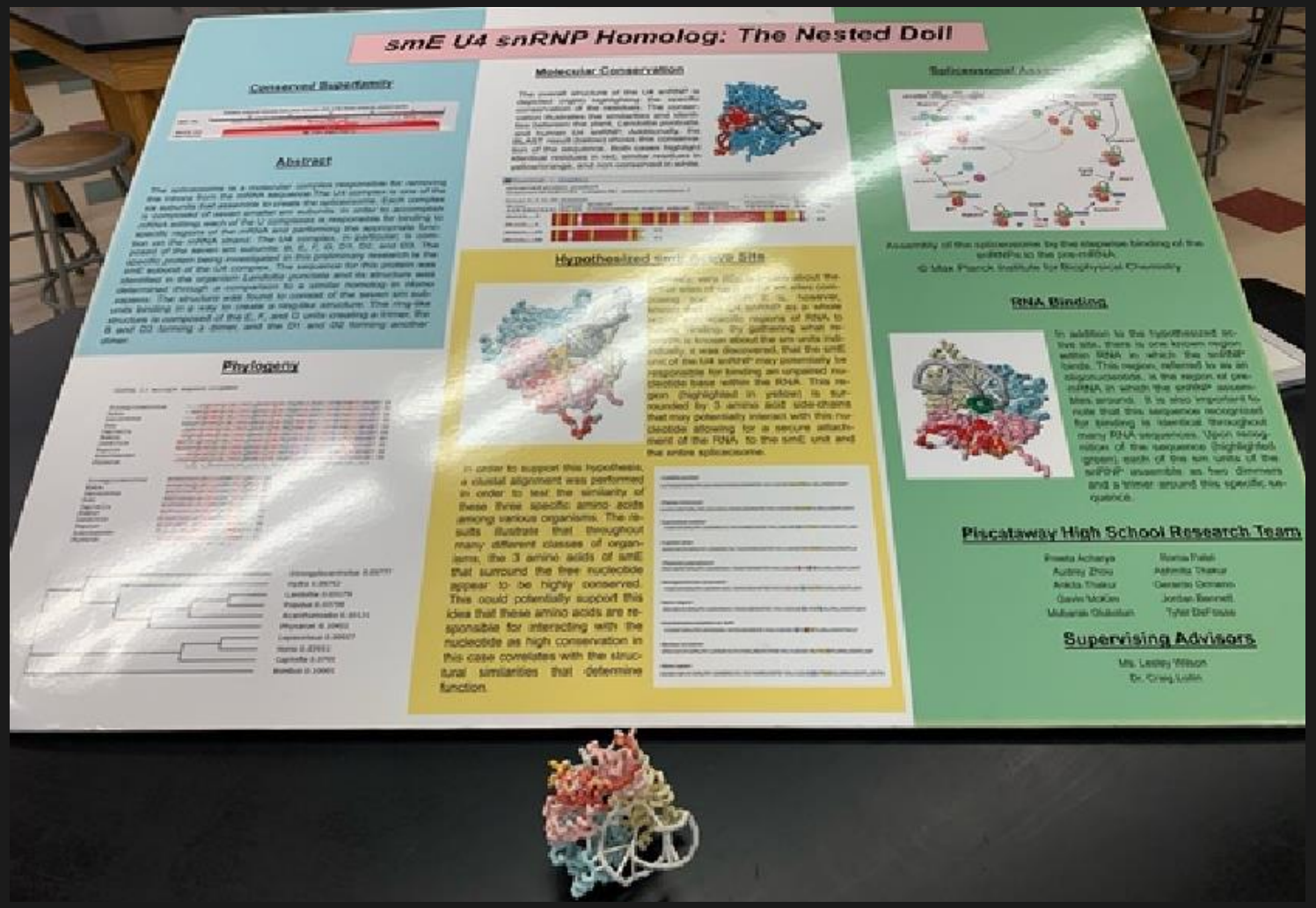
I am familiar with the PDB due to my involvement in the Waksman Student Scholars Program - WSSP https://wssp.rutgers.edu/

Originally 2Y9A – Now Obsolete and entered under 4WZJ – Spliceosomal U4 snRNP core domain

https://www.rcsb.org/structure/4WZJ

My WSSP (Piscataway High School, NJ) students identified one of the seven “ring” proteins of this snRNP complex and when we went to model it using jMol, we discovered there was RNA within the structure. We decided to go ahead a model it, but needed permission from WSSP to go ahead with this since they had not modelled anything with RNA to date...so we were the first to do that within the program.

Honestly, we were very proud of ourselves!



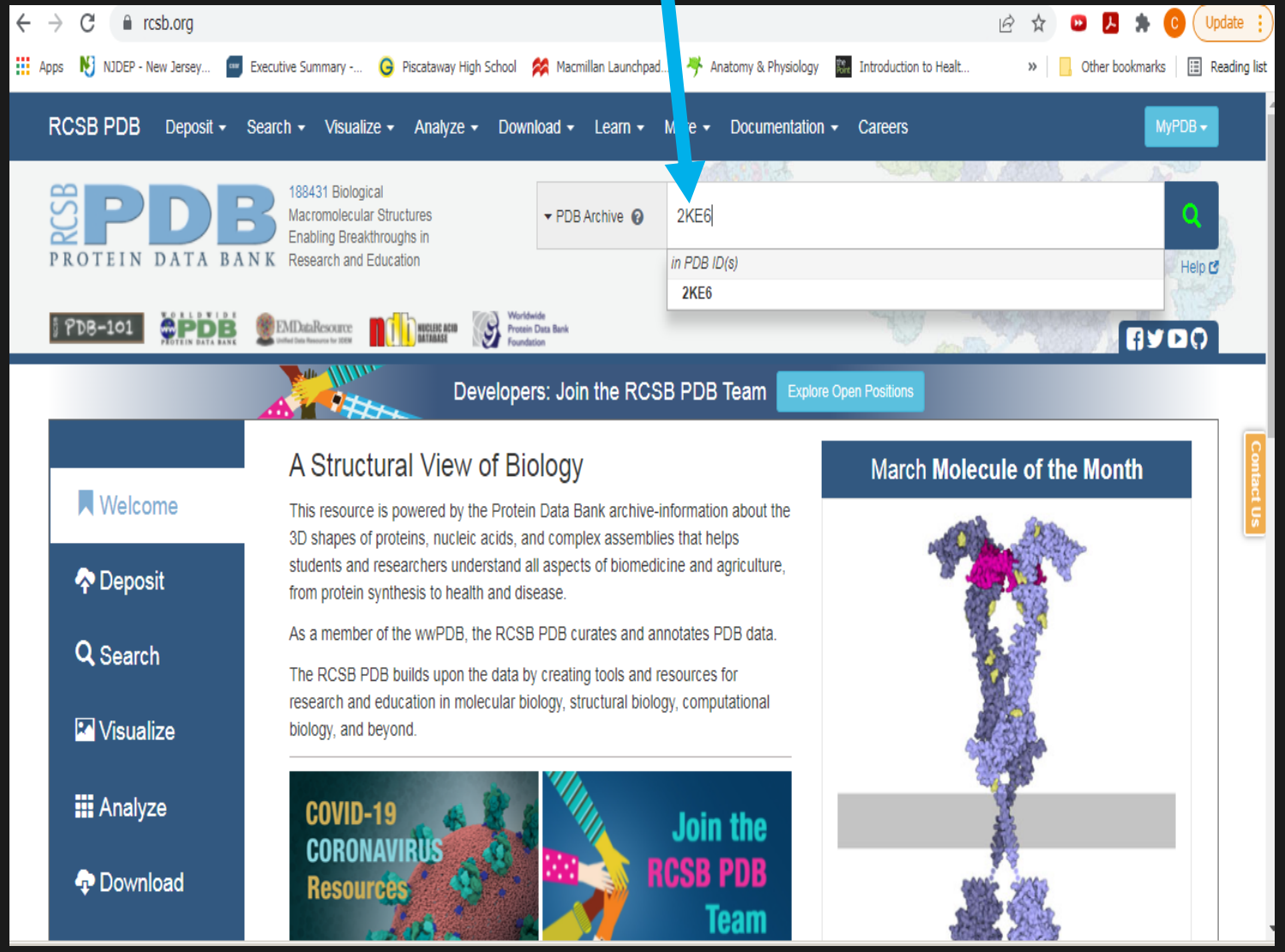
So how do we use PDB?

Every molecular model (atomic coordinate file) in the Protein Data Bank (PDB) has a unique accession or identification code. These codes are always 4 characters in length. The first character is a numeral in the range 1-9, while the last three characters can be either numerals (in the range 0-9) or letters (in the range A-Z in the Latin alphabet). Plans for an expanded identification code system that handle more entries have been announced.

Just paste in the PDB Accession # into this field and usually it will show you a drop down of the Accession # - just click it.

This will take you to the main PDB page for that entry.

This is where you will find all the relevant details and files.

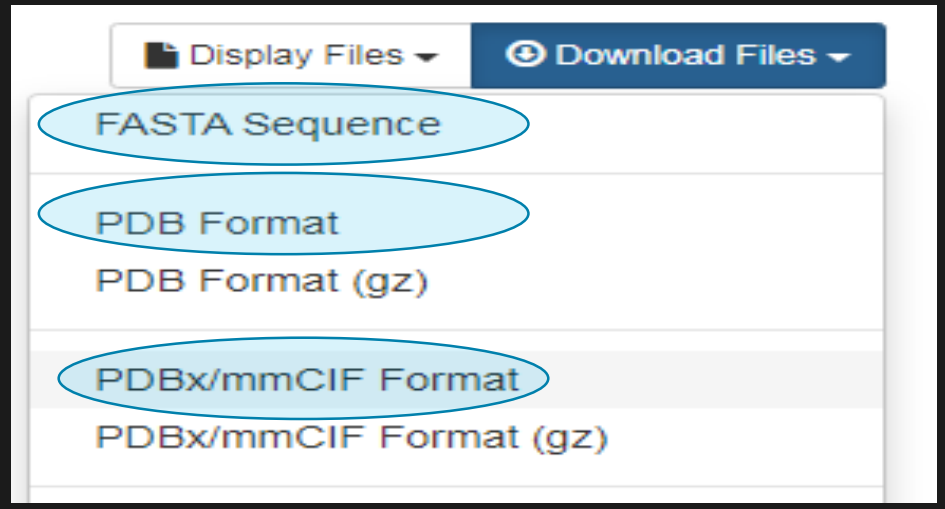


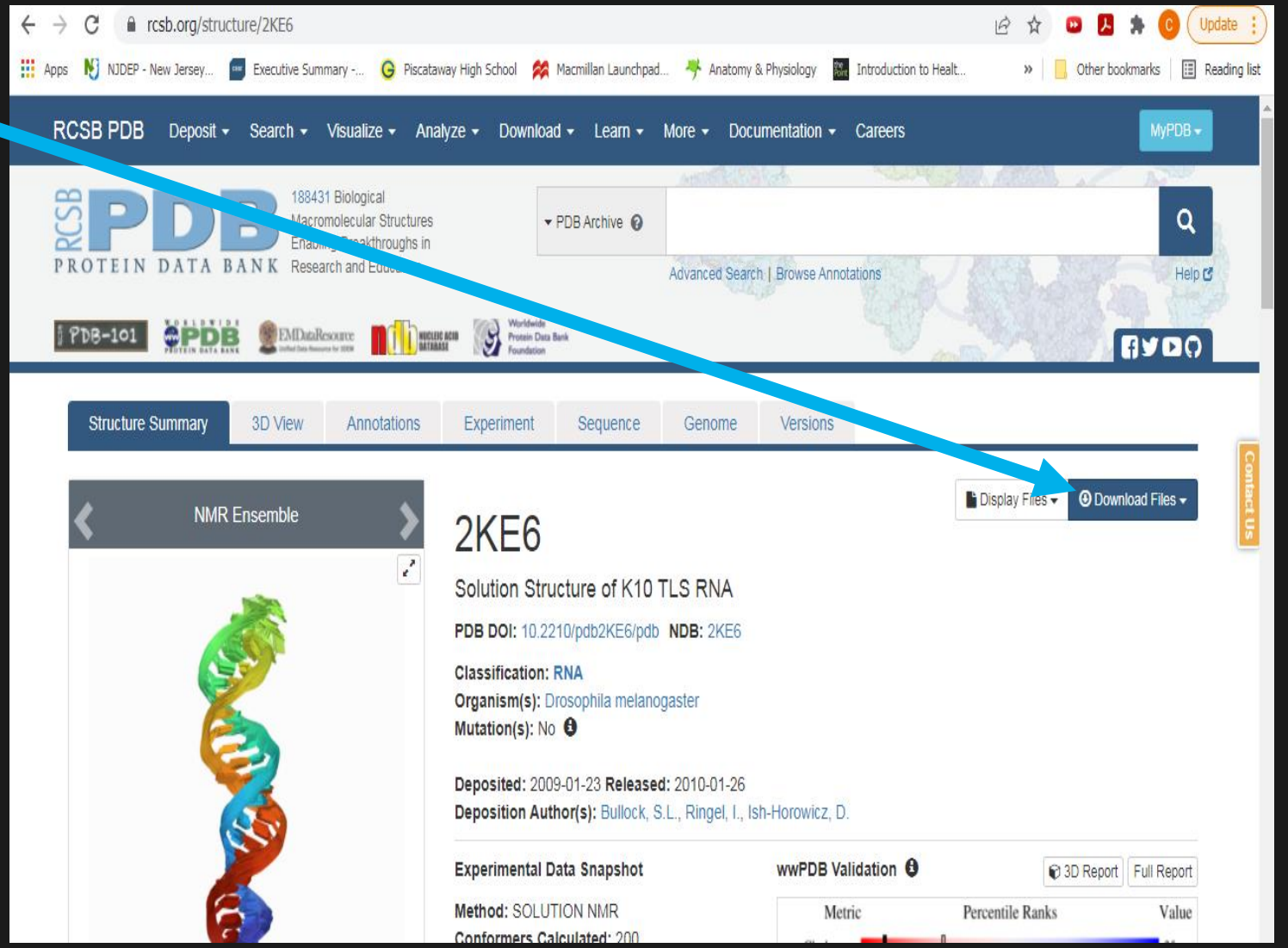
So how do we use PDB?

These are the files associated with the PDB entry.

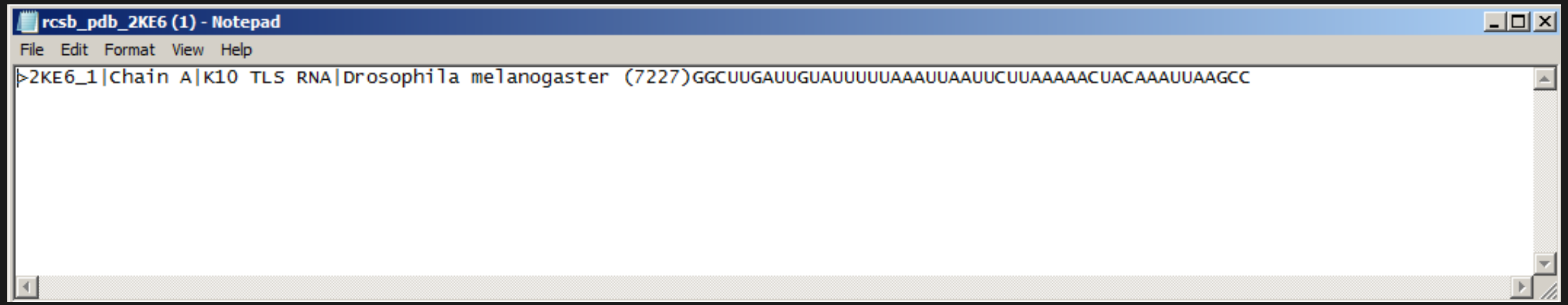
You will want the FASTA sequence file, the PDB format file, possibly the CIF file...

I recommend you download them into a folder for use and possible later research.





Files – FASTA Sequence file



Files – PDB Format file


```
HEADER    RNA                                     23-JAN-09   2KE6
TITLE     SOLUTION STRUCTURE OF K10 TLS RNA
COMPND    MOL_ID: 1;
COMPND   2 MOLECULE: K10 TLS RNA;
COMPND   3 CHAIN: A;
COMPND   4 ENGINEERED: YES;
COMPND   5 OTHER_DETAILS: K10 TRANSPORT AND LOCALISATION SIGNAL (TLS)
SOURCE    MOL_ID: 1;
SOURCE   2 SYNTHETIC: YES;
SOURCE   3 ORGANISM_SCIENTIFIC: DROSOPHILA MELANOGASTER;
SOURCE   4 ORGANISM_COMMON: FRUIT FLY;
SOURCE   5 ORGANISM_TAXID: 7227;
SOURCE   6 OTHER_DETAILS: PREPARED BY IN VITRO TRANSCRIPTION USING T7
KEYWDS    RNA TRANSPORT, RNA HAIRPIN, RNA
EXPDTA    SOLUTION NMR
NUMMDL    12
AUTHOR    S.L.BULLOCK,I.RINGEL,D.ISH-HOROWICZ
REVDAT   5   16-MAR-22 2KE6    1       REMARK
REVDAT   4   16-JUN-10 2KE6    1       JRNL
REVDAT   3   26-MAY-10 2KE6    1       JRNL
REVDAT   2   19-MAY-10 2KE6    1       JRNL   REMARK COMPND AUTHOR
REVDAT   1   26-JAN-10 2KE6    0
JRNL        AUTH   S.L.BULLOCK,I.RINGEL,D.ISH-HOROWICZ,P.J.LUKAVSKY
JRNL        TITL   A'-FORM RNA HELICES ARE REQUIRED FOR CYTOPLASMIC MRNA
JRNL        TITL 2 TRANSPORT IN DROSOPHILA
JRNL        REF    NAT.STRUCT.MOL.BIOL.          V.  17   703 2010
JRNL        REFN                   ISSN 1545-9993
JRNL        PMID   20473315
JRNL        DOI    10.1038/NSMB.1813
REMARK   2
```

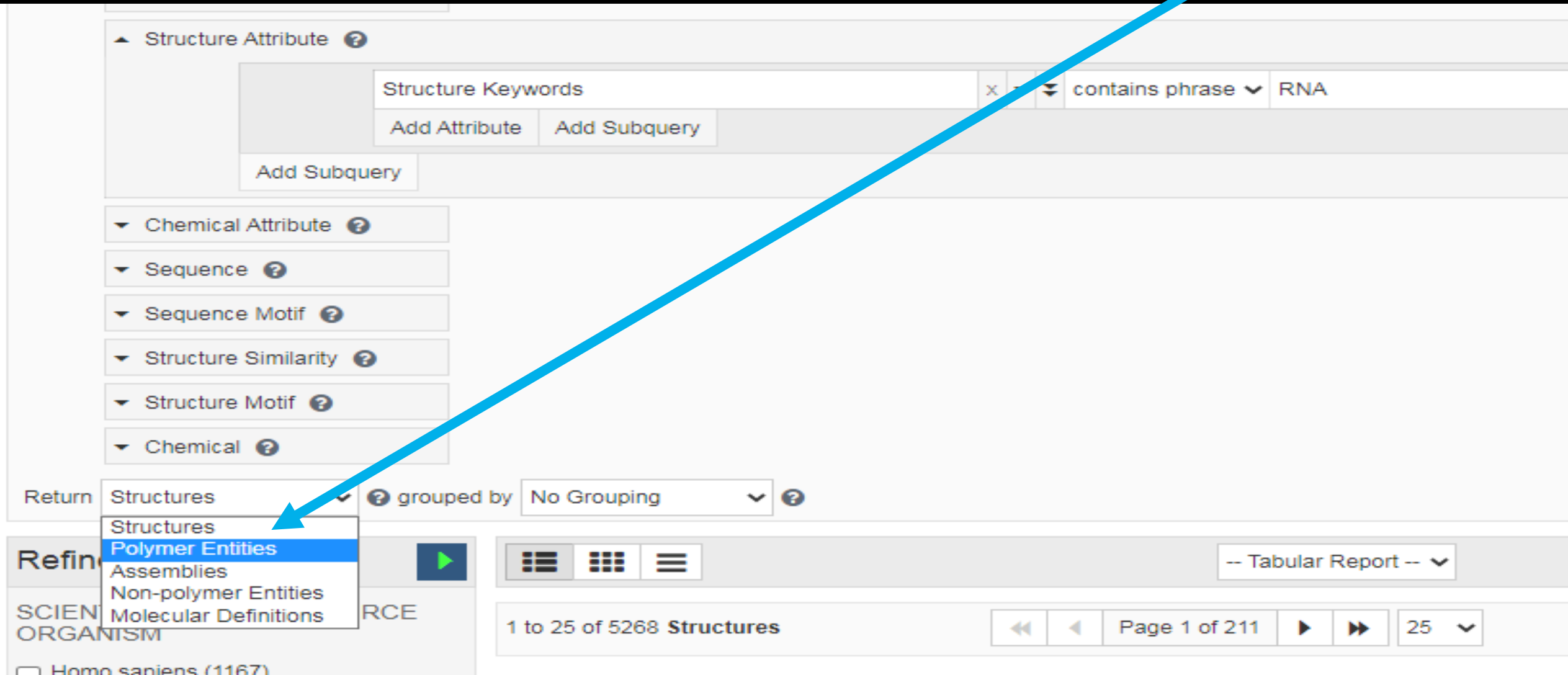
```
ORIGX2      0.000000  1.000000  0.000000        0.00000
ORIGX3      0.000000  0.000000  1.000000        0.00000
SCALE1      1.000000  0.000000  0.000000        0.00000
SCALE2      0.000000  1.000000  0.000000        0.00000
SCALE3      0.000000  0.000000  1.000000        0.00000
MODEL        1
ATOM      1  O5'   G A   1      -5.468  -1.296  32.261  1.00  0.00           O
ATOM      2  C5'   G A   1      -4.486  -0.267  32.420  1.00  0.00           C
ATOM      3  C4'   G A   1      -3.080  -0.845  32.551  1.00  0.00           C
ATOM      4  O4'   G A   1      -3.049  -1.792  33.625  1.00  0.00           O
ATOM      5  C3'   G A   1      -2.767  -1.610  31.277  1.00  0.00           C
ATOM      6  O3'   G A   1      -1.926  -0.827  30.423  1.00  0.00           O
ATOM      7  C2'   G A   1      -2.016  -2.848  31.740  1.00  0.00           C
ATOM      8  O2'   G A   1      -0.610  -2.730  31.506  1.00  0.00           O
ATOM      9  C1'   G A   1      -2.322  -2.970  33.231  1.00  0.00           C
ATOM     10  N9    G A   1      -3.091  -4.201  33.500  1.00  0.00           N
ATOM     11  C8    G A   1      -4.426  -4.349  33.764  1.00  0.00           C
ATOM     12  N7    G A   1      -4.803  -5.585  33.943  1.00  0.00           N
ATOM     13  C5    G A   1      -3.628  -6.316  33.786  1.00  0.00           C
ATOM     14  C6    G A   1      -3.402  -7.719  33.868  1.00  0.00           C
ATOM     15  O6    G A   1      -4.221  -8.606  34.098  1.00  0.00           O
ATOM     16  N1    G A   1      -2.068  -8.034  33.645  1.00  0.00           N
ATOM     17  C2    G A   1      -1.077  -7.116  33.377  1.00  0.00           C
ATOM     18  N2    G A   1       0.144  -7.609  33.183  1.00  0.00           N
ATOM     19  N3    G A   1      -1.280  -5.801  33.300  1.00  0.00           N
ATOM     20  C4    G A   1      -2.575  -5.473  33.515  1.00  0.00           C
ATOM     21  H5'   G A   1      -4.718   0.310  33.315  1.00  0.00           H
ATOM     22 H5''   G A   1      -4.520   0.394  31.553  1.00  0.00           H
ATOM     23  H4'   G A   1      -2.349  -0.054  32.716  1.00  0.00           H
ATOM     24  H3'   G A   1      -3.686  -1.891  30.761  1.00  0.00           H
```

So how did I make it a **new one**?

- Go to PDB and search for anything that has RNA, but I ran into problems immediately.
- Many PDB entries have RNA and Protein and the PDB Format files have ALL the coordinate data for both (ALL) and so it will not work in ETERNA when trying to upload the 3D file in PuzzleMaker. (basically it needs to be just 1 sequence).
- My options at this point are to edit the PDB Format file and delete away any non-relevant sequence data – either from protein or other RNA or even hetero atoms like HOH (water).
- I tried this but when saving the file it cannot be saved as a PDB file extension (or at least I can't do that) – soooo, this may be a dead end!!!
- So the other option is to search for polymer entities, which restricts your search to entries with just 1 type of sequence.

So how did I make it a **new one**?

- Within PDB, search for RNA, but restrict your search to Polymer Entities



- [ ] DNA (841)
- [ ] NA-hybrid (157)
- [ ] Other (4)

REFINEMENT RESOLUTION (Å)

- [ ] 0.5 - 1.0 (24)
- [ ] 1.0 - 1.5 (487)
- [ ] 1.5 - 2.0 (1378)
- [ ] 2.0 - 2.5 (1699)
- [ ] 2.5 - 3.0 (2403)
- [ ] 3.0 - 3.5 (2295)
- [ ] 3.5 - 4.0 (1662)
- [ ] 4.0 - 4.5 (644)
- [ ] > 4.5 (766)

RELEASE DATE

- [ ] 1975 - 1979 (2)
- [ ] 1985 - 1989 (14)
- [ ] 1990 - 1994 (69)
- [ ] 1995 - 1999 (337)
- [ ] 2000 - 2004 (784)
- [ ] 2005 - 2009 (2101)
- [ ] 2010 - 2014 (2383)
- [ ] 2015 - 2019 (4223)
- [ ] 2020 - 2024 (2726)

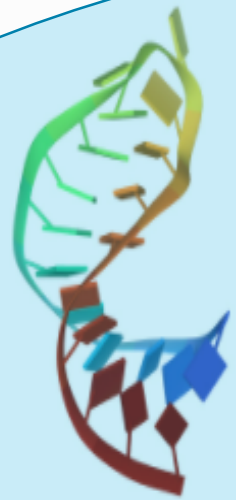
3D View

DUPLEX CONTAINING TWO G(ANTI).A(ANTI) BASE-PAIRS

Leonard, G.A., McAuley-Hecht, K.E., Ebel, S., Lough, D.M., Brown, T., Hunter, W.N.

(1994) Structure **2**: 483-494

Released	1994-05-31
Method	X-RAY DIFFRACTION 1.8 Å
Chain IDs	A, B
Macromolecule	RNA (5'-R(*CP*GP*CP*GP*AP*AP*UP*UP*AP*GP*CP*G)-3')



3D View

17RA: Entity 1

Download File | View File

BRANCHPOINT HELIX FROM YEAST AND BINDING SITE FOR PHAGE GA/MS2 COAT PROTEINS, NMR, 12 STRUCTURES

Nikonowicz, E.P., Smith, J.S.

(1998) Biochemistry **37**: 13486-13498

Released	1999-04-20
Method	SOLUTION NMR
Chain ID	A
Organism	
Macromolecule	RNA

1A3M: Entity 1

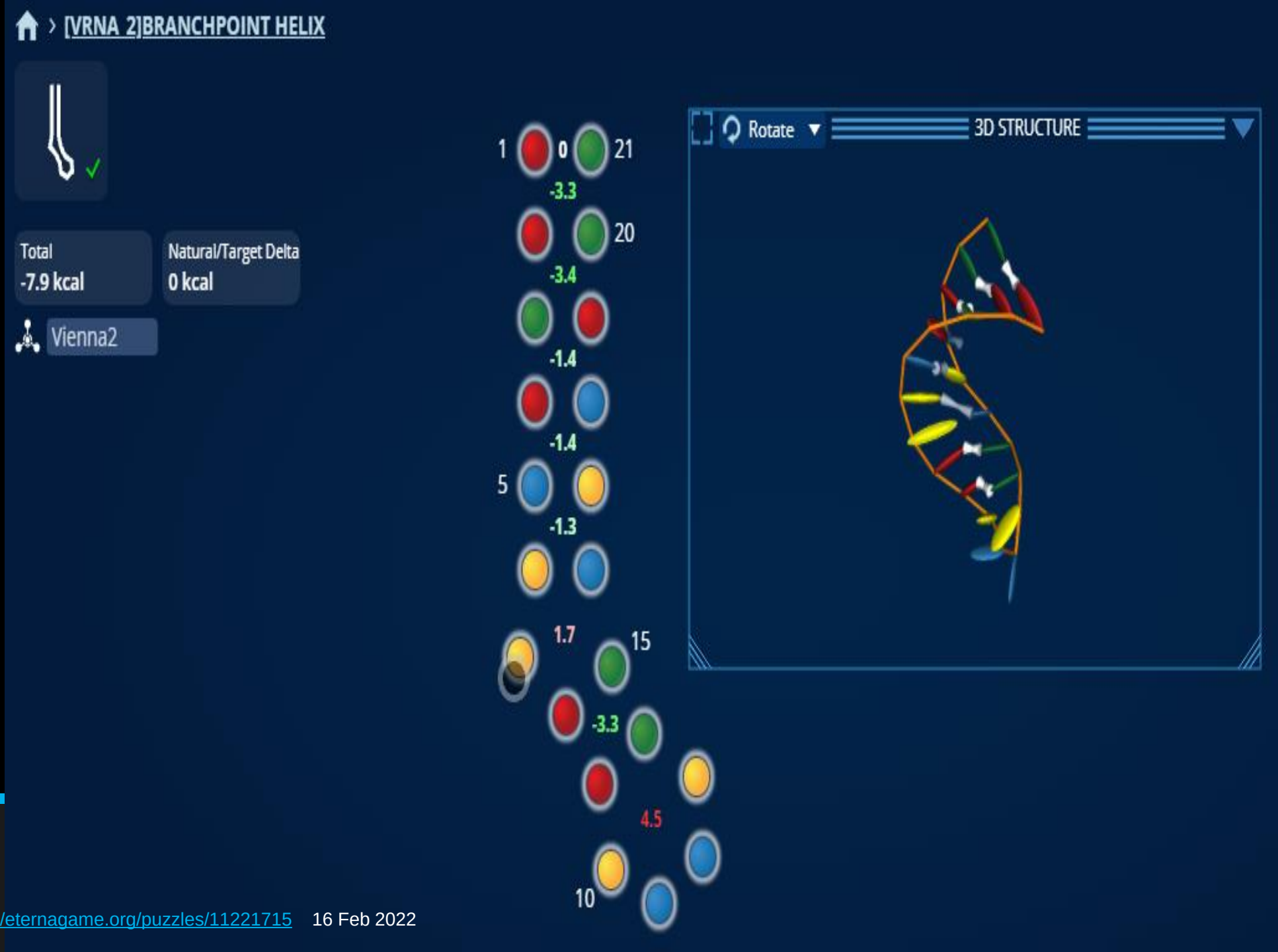
Download File | View File

PAROMOMYCIN BINDING INDUCES A LOCAL CONFORMATIONAL CHANGE IN THE A SITE OF 16S RRNA, NMR, 20 STRUCTURES

About the Puzzle

@DigitalEmbrace Suggested I create the first 3D player made puzzle. Obtained from PDB entry 17RA - BRANCHPOINT HELIX FROM YEAST AND BINDING SITE FOR PHAGE GA/MS2 COAT PROTEINS, NMR, 12 STRUCTURES

This is a very small, very simple RNA entity that I thought could have a clean PDB file so it would work in the Eterna 3D model. It can be done!

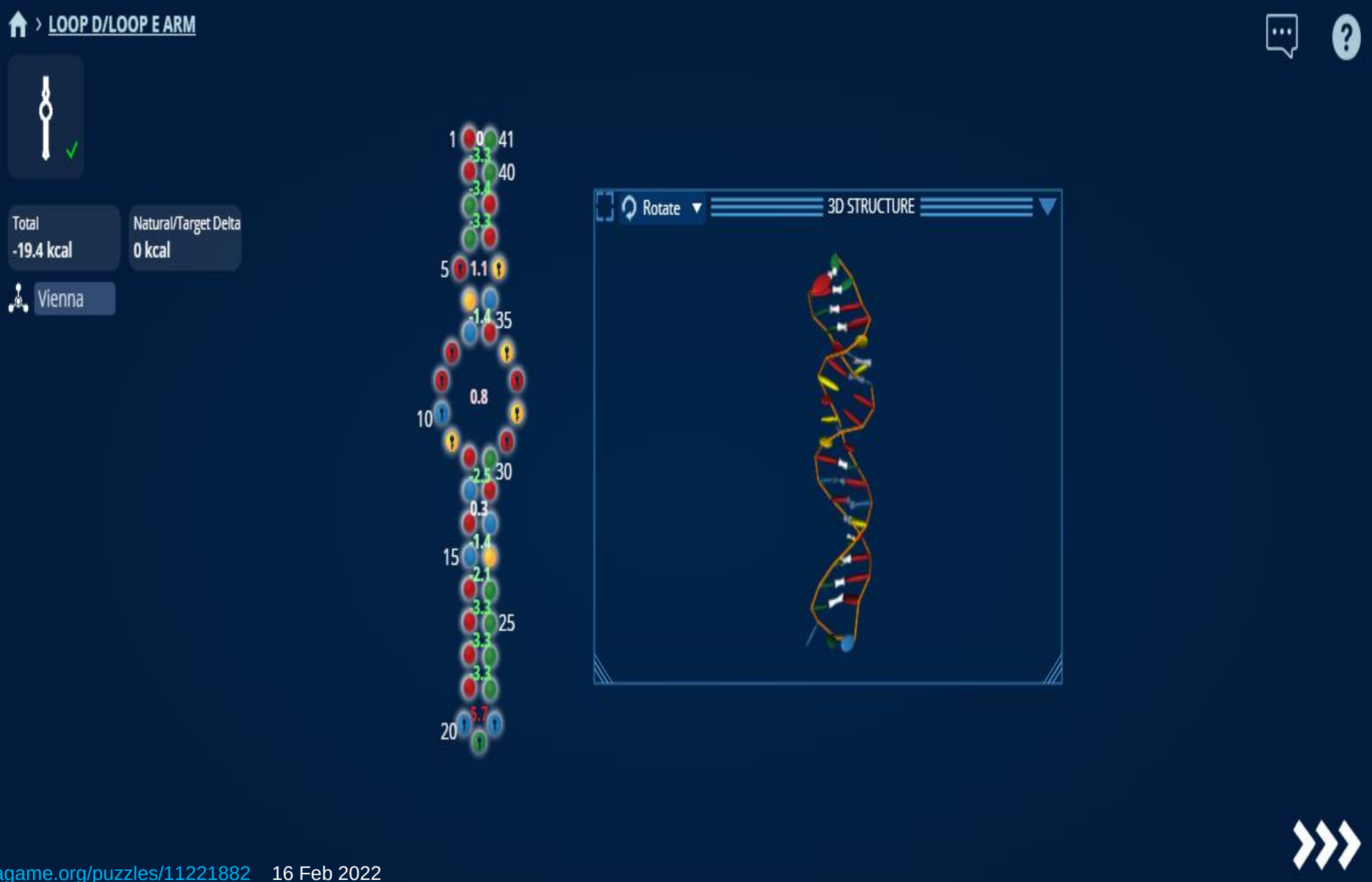


1st - [VRNA_2]BRANCHPOINT HELIX https://eternagame.org/puzzles/11221715 16 Feb 2022

About the Puzzle

PDB Entry 1A51 - LOOP D/LOOP E ARM OF E. COLI 5S RRNA, NMR, 9 STRUCTURES

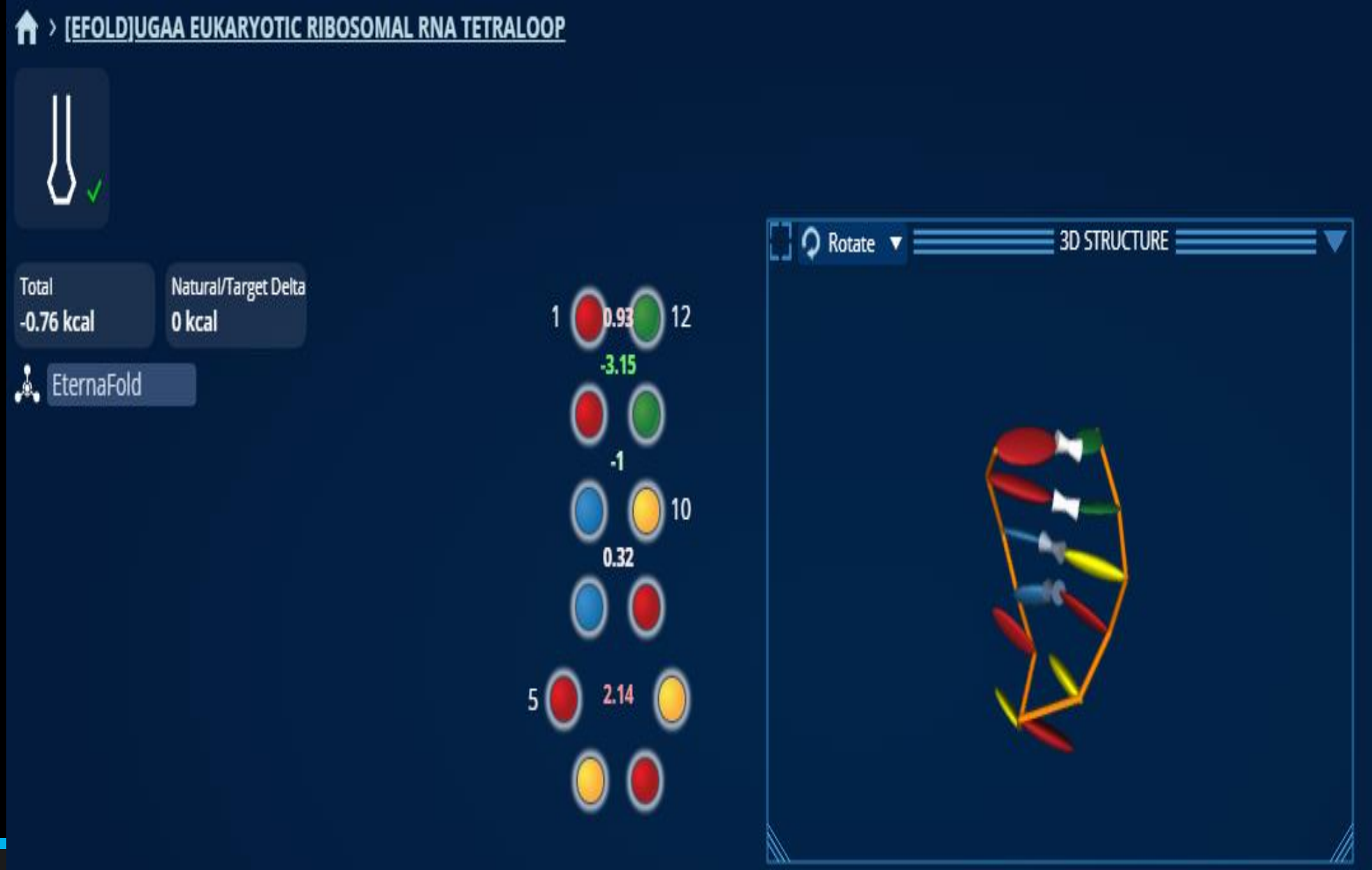
Locked the unpaired bases per the native sequence from PDB FASTA



2nd - LOOP D/LOOP E ARM https://eternagame.org/puzzles/11221882 16 Feb 2022

About the Puzzle

PDB Entry 1AFX - UGAA EUKARYOTIC RIBOSOMAL RNA TETRALOOP, NMR, 13 STRUCTURES



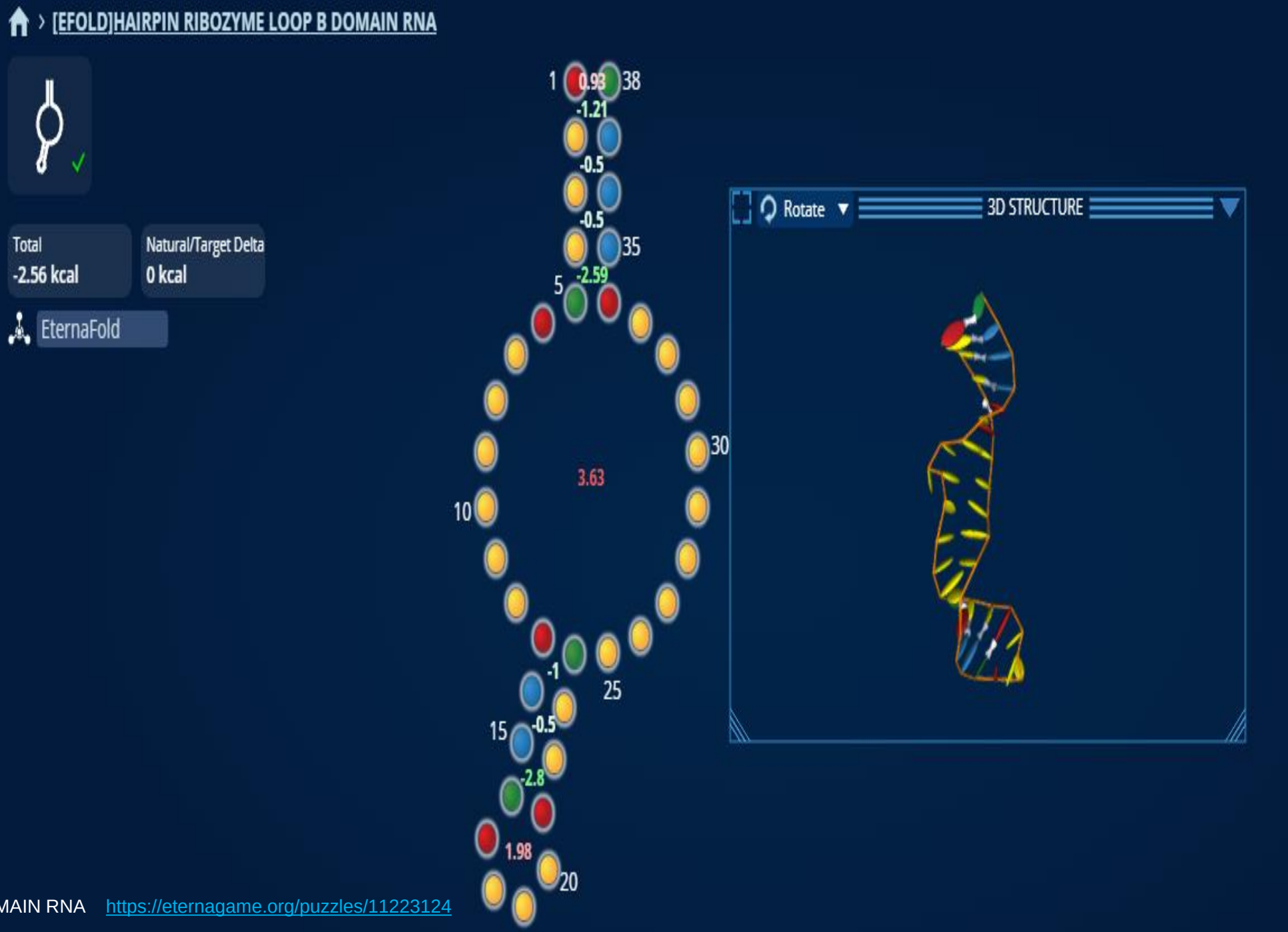
3rd - [EFOLD]UGAA EUKARYOTIC RIBOSOMAL RNA TETRALOOP https://eternagame.org/puzzles/11223109
17 Feb 2022

About the Puzzle

PDB Entry 1B36 - SOLUTION STRUCTURE OF THE HAIRPIN RIBOZYME LOOP B DOMAIN RNA, NMR, 10 STRUCTURES

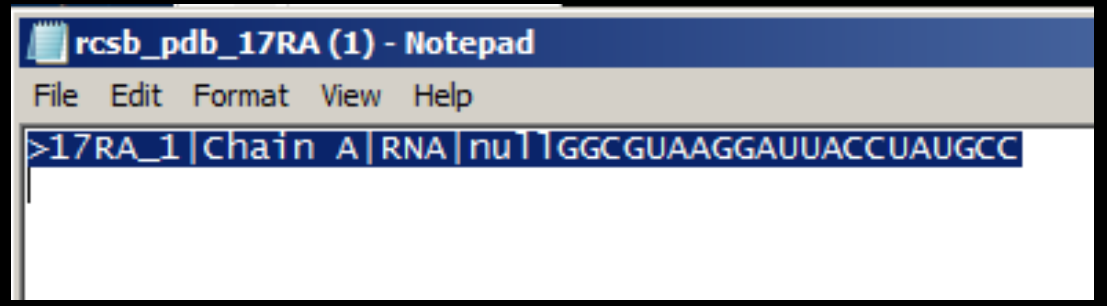
https://www.nature.com/articles/nsb0399_212

From the Abstract: "Here we present the solution structure of the loop B domain of the hairpin ribozyme, which contains most of the catalytically essential nucleotides. The 38-nucleotide domain contains a 16-nucleotide internal loop that forms one of the largest non-Watson–Crick segments of base pairing thus far determined by either NMR or crystallography. "



How do I get the Dot Bracket Structure for this sequence...?

RNA Fold
http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi



Results for minimum free energy prediction

The optimal secondary structure in dot-bracket notation wi
[color by base-pairing probability | color by positional entr

```
1       GGCGUAAGGAUUACCUAUGCC

1       ((((((.((....)))))))))
```

You can download the minimum free energy (MFE) structu
server.

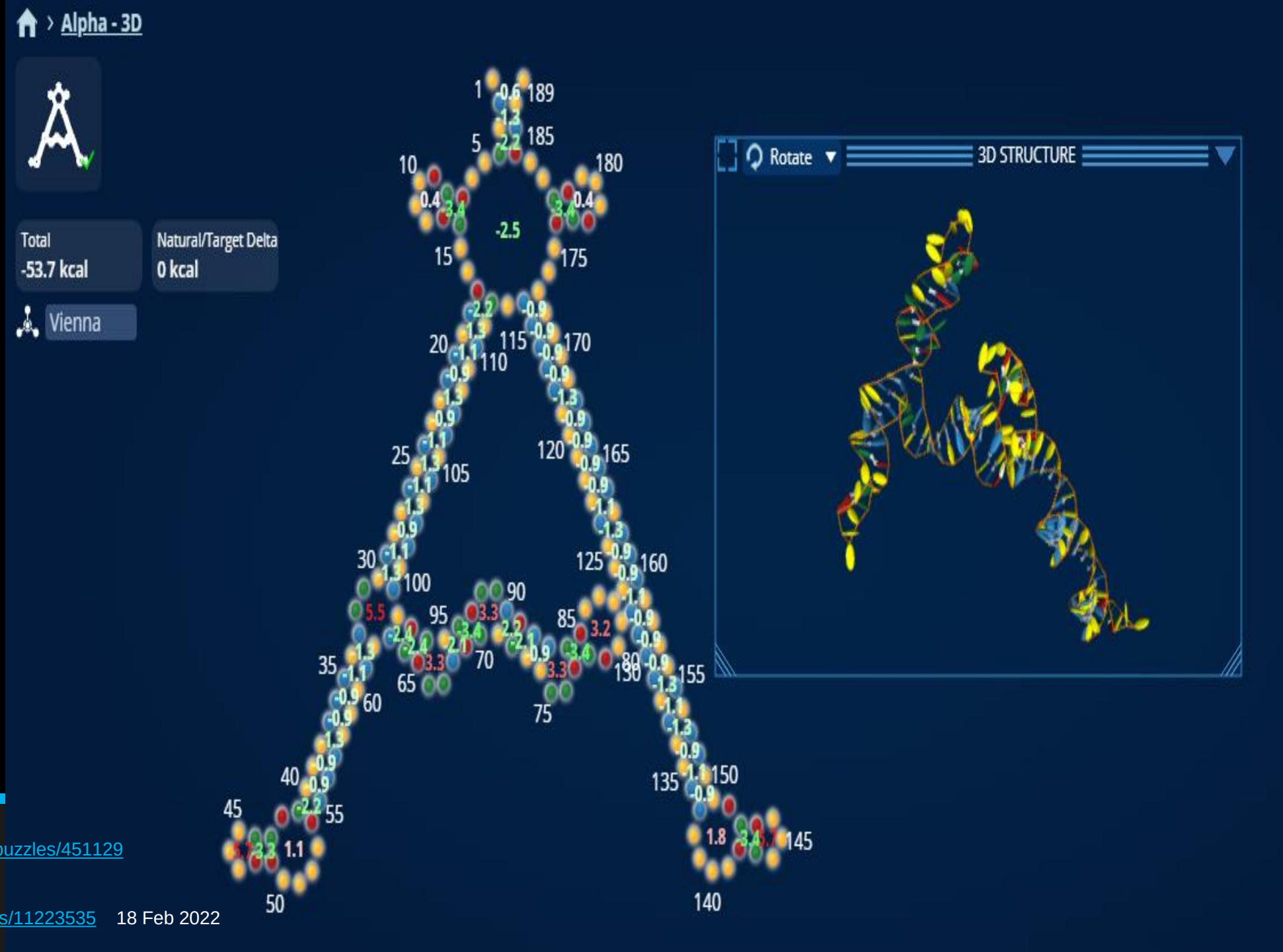
- CAN I DO THIS WITH MY EXISTING PUZZLES? And therefore with any other puzzle in ETERNA...
- OKAY, I can get the Dot Bracket Structure for my created puzzles (RNA Fold), but my problem now is created puzzles don’t have PDB Format files to create the 3D info for PuzzleMaker...sad face...stop now...never
- Natural question...how do I create a PDB Format file?

About the Puzzle

This is awesome! Thanks owed to several people in the forum, but a little digging brought me to this site: https://rnacomposer.cs.put.poznan.pl/

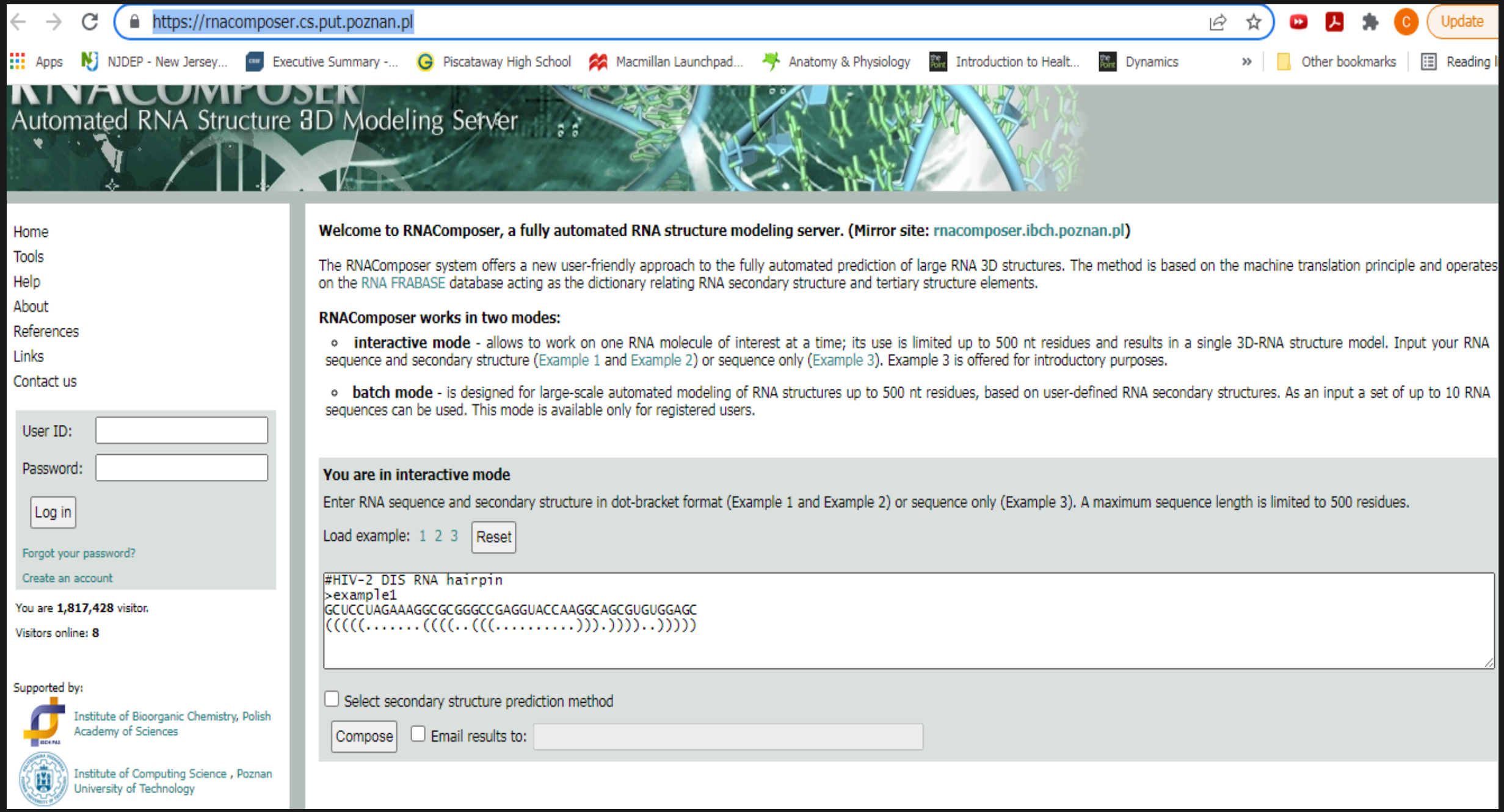
With the sequence from my puzzle and the dot-bracket code from puzzle maker, I was able to create a PBD text file which works perfectly in jMol and in this Eterna 3D puzzle.

What's even cooler, is that in 3D it looks a lot like the letter A.



Original Alpha Puzzle https://eternagame.org/puzzles/451129

5th - Alpha - 3D https://eternagame.org/puzzles/11223535 18 Feb 2022


https://rnacomposer.cs.put.poznan.pl
Update
Apps
NJDEP - New Jersey...
Executive Summary -...
Piscataway High School
Macmillan Launchpad...
Anatomy & Physiology
Introduction to Healt...
Dynamics
Other bookmarks
Reading l
RNACOMPOSER
Automated RNA Structure 3D Modeling Server
Home
Tools
Help
About
References
Links
Contact us
User ID:
Password:
Log in
Forgot your password?
Create an account
You are 1,817,428 visitor.
Visitors online: 8
Supported by:
Institute of Bioorganic Chemistry, Polish Academy of Sciences
Institute of Computing Science , Poznan University of Technology
Welcome to RNAComposer, a fully automated RNA structure modeling server. (Mirror site: rnacomposer.ibch.poznan.pl)
The RNAComposer system offers a new user-friendly approach to the fully automated prediction of large RNA 3D structures. The method is based on the machine translation principle and operates on the RNA FRABASE database acting as the dictionary relating RNA secondary structure and tertiary structure elements.
RNAComposer works in two modes:
interactive mode - allows to work on one RNA molecule of interest at a time; its use is limited up to 500 nt residues and results in a single 3D-RNA structure model. Input your RNA sequence and secondary structure (Example 1 and Example 2) or sequence only (Example 3). Example 3 is offered for introductory purposes.
batch mode - is designed for large-scale automated modeling of RNA structures up to 500 nt residues, based on user-defined RNA secondary structures. As an input a set of up to 10 RNA sequences can be used. This mode is available only for registered users.
You are in interactive mode
Enter RNA sequence and secondary structure in dot-bracket format (Example 1 and Example 2) or sequence only (Example 3). A maximum sequence length is limited to 500 residues.
Load example: 1 2 3
Reset
#HIV-2 DIS RNA hairpin
>example1
GCUCCUAGAAAGGCGCGGGCCGAGGUACCAAGGCAGCGUGUGGAGC
(((((.......((((..(((..........))).)))))..)))))
Select secondary structure prediction method
Compose
Email results to:

RNA COMPOSER https://rnacomposer.cs.put.poznan.pl/

Info for RNA Composer site:

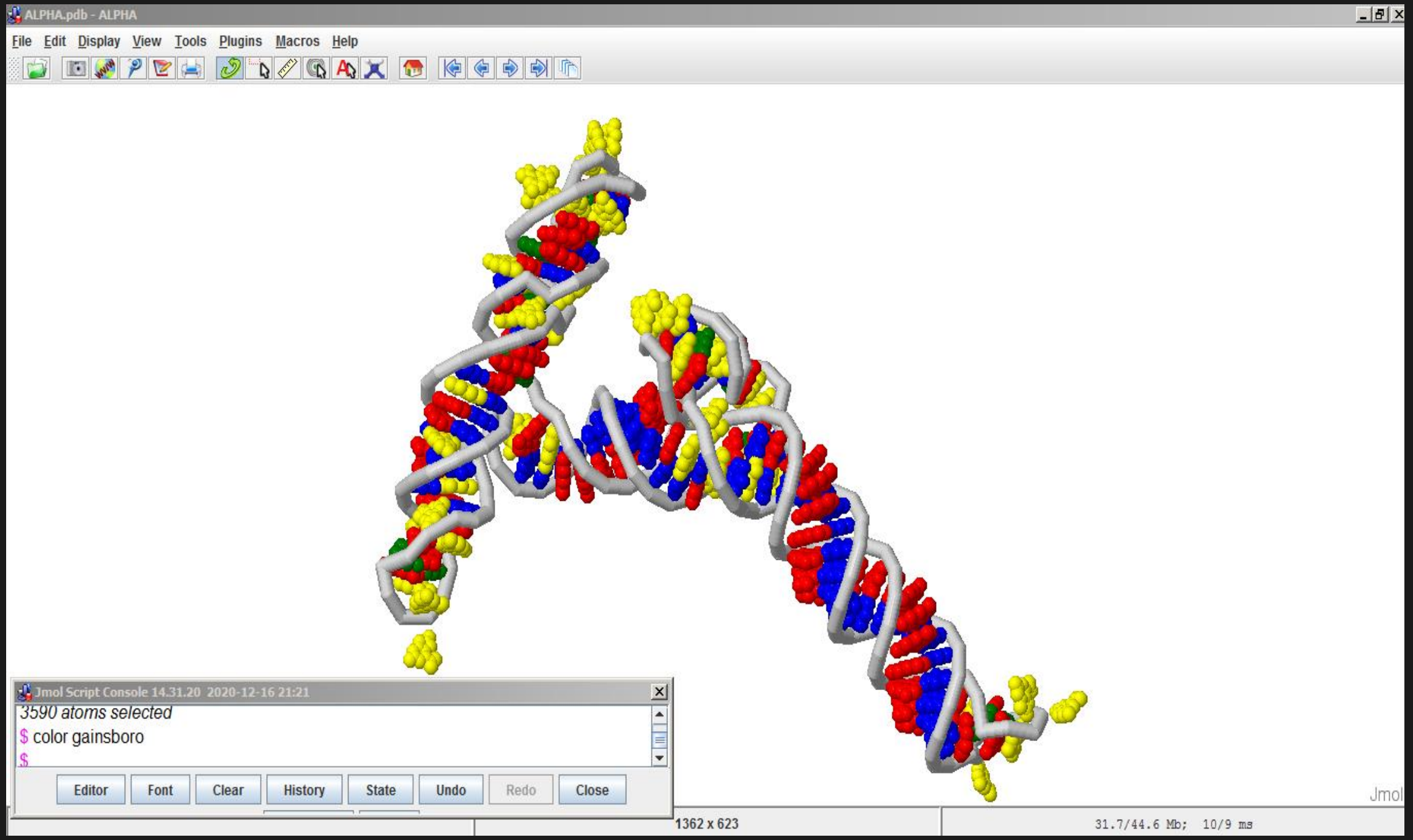
Alpha letter in clollin series Eterna

>ALPHA

AUGCAAGUGAAAGCAAGUUUUUUUUUUUUAAGUUUAUGUGAGCAAAGCGAAGUAUGUGGAUGGUAAUCAGCGGAAUGGAAAAACACUGUAAUGAGCCGAGGGGGGGGG
GAUAUAUUUUUUUUUUUUUUUUUUUUUGAAGGCAAAGCAGGGGGGGGGGGGGGGGGGGGGGUGAAGAGAAAUCAAGUAA

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

```
MODEL        1
ATOM      1  HO5'   A A   1      -0.433  -0.197  -1.235  1.00  0.00           H
ATOM      2  O5'    A A   1      -0.072   0.491  -0.669  1.00  0.00           O
ATOM      3  C5'    A A   1       1.303   0.175  -0.436  1.00  0.00           C
ATOM      4  H5'    A A   1       1.930   1.007  -0.759  1.00  0.00           H
ATOM      5  H5''   A A   1       1.576  -0.713  -1.004  1.00  0.00           H
ATOM      6  C4'    A A   1       1.549  -0.091   1.031  1.00  0.00           C
ATOM      7  H4'    A A   1       0.605   0.059   1.556  1.00  0.00           H
ATOM      8  O4'    A A   1       2.089  -1.436   1.177  1.00  0.00           O
ATOM      9  C1'    A A   1       3.156  -1.424   2.108  1.00  0.00           C
ATOM     10  H1'    A A   1       2.991  -2.254   2.797  1.00  0.00           H
ATOM     11  N9     A A   1       4.402  -1.671   1.384  1.00  0.00           N
ATOM     12  C4     A A   1       5.594  -2.080   1.931  1.00  0.00           C
ATOM     13  N3     A A   1       5.851  -2.340   3.225  1.00  0.00           N
ATOM     14  C2     A A   1       7.119  -2.711   3.386  1.00  0.00           C
ATOM     15  H2     A A   1       7.411  -2.943   4.410  1.00  0.00           H
ATOM     16  N1     A A   1       8.086  -2.842   2.469  1.00  0.00           N
ATOM     17  C6     A A   1       7.794  -2.572   1.179  1.00  0.00           C
ATOM     18  N6     A A   1       8.758  -2.701   0.267  1.00  0.00           N
ATOM     19  H61    A A   1       8.560  -2.503  -0.703  1.00  0.00           H
ATOM     20  H62    A A   1       9.682  -2.992   0.552  1.00  0.00           H
ATOM     21  C5     A A   1       6.483  -2.169   0.876  1.00  0.00           C
ATOM     22  N7     A A   1       5.863  -1.825  -0.318  1.00  0.00           N
ATOM     23  C8     A A   1       4.634  -1.540   0.037  1.00  0.00           C
ATOM     24  H8     A A   1       3.873  -1.232  -0.665  1.00  0.00           H
ATOM     25  C2'    A A   1       3.117  -0.070   2.813  1.00  0.00           C
ATOM     26  H2'    A A   1       4.114   0.255   3.118  1.00  0.00           H
ATOM     27  O2'    A A   1       2.209  -0.146   3.896  1.00  0.00           O
ATOM     28  HO2'   A A   1       1.738   0.689   3.920  1.00  0.00           H
ATOM     29  C3'    A A   1       2.577   0.815   1.698  1.00  0.00           C
```


ALPHA.pdb - ALPHA
File Edit Display View Tools Plugins Macros Help
Jmol Script Console 14.31.20 2020-12-16 21:21
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$ color gainsboro
$
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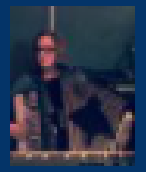
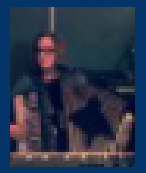
Jmol
1362 x 623
31.7/44.6 Mb; 10/9 ms



DigitalEmbrace
19 Feb 2022

The 3D model must be purely a prediction since you used RNAComposer and no natural RNA would have straight helices. Great work!

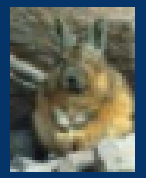


DigitalEmbrace
18 Feb 2022

Is the PDB file based on the known 3D structure or is it a prediction based on the dot-bracket and sequence?

jnicol
18 Feb 2022

Nice!



Anamfija
18 Feb 2022

Whoa, that's super cool! I had seen RNApdbee before (which takes in a PDB ID or 3D structure and changes it into dot-bracket notation), but I hadn't come across RNAComposer yet. So many possibilities . . .

Conclusion

- 3D Puzzles can be made by anyone with a fairly straight forward series of steps...if you run into difficulty; I am more than happy to try and help!
- Try one and see!
- https://forum.eternagame.org/t/testing-eterna3d-functionality/4144 - *DigitalEmbrace*

Problems

- Puzzles that we create are rarely representations of biologically valid sequences and are thus **NOT** verified regarding folding (3D)

How to control 3D puzzles

I just tried some things that work with Jmol and realized that you can use the shift button with the mouse and it will pan. So all three options for moving would be directly accessible if rotate was the default.

(For me anyway)

- Shift and left click = pan.
- Left click alone = rotate.
- Scroll wheel = zoom.

Try it out!

QUESTIONS?

Acknowledgments: So many Eterna players have helped me in ways they may never know; whether it comes to solving a puzzle or learning something new. Someone even recently helped my 5yr old daughter in the chat while she was playing on the site. Thanks to all, especially:

DigitalEmbrace, lroppy, Eli Fisker, Anamfija, Astromon, jnicol, ETERNA DEV team and many others not mentioned...