

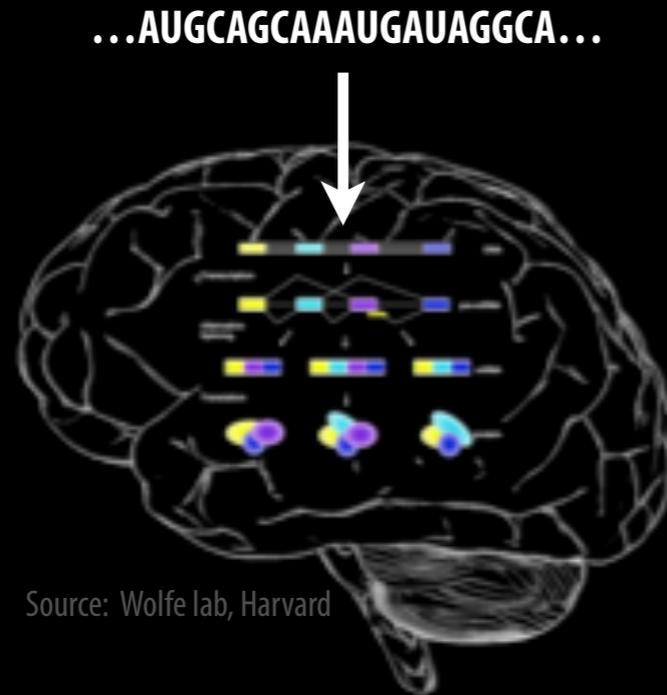
Biomolecule Design Rules from an **Internet-scale Videogame** with Experiments

Rhiju Das, Ph.D.

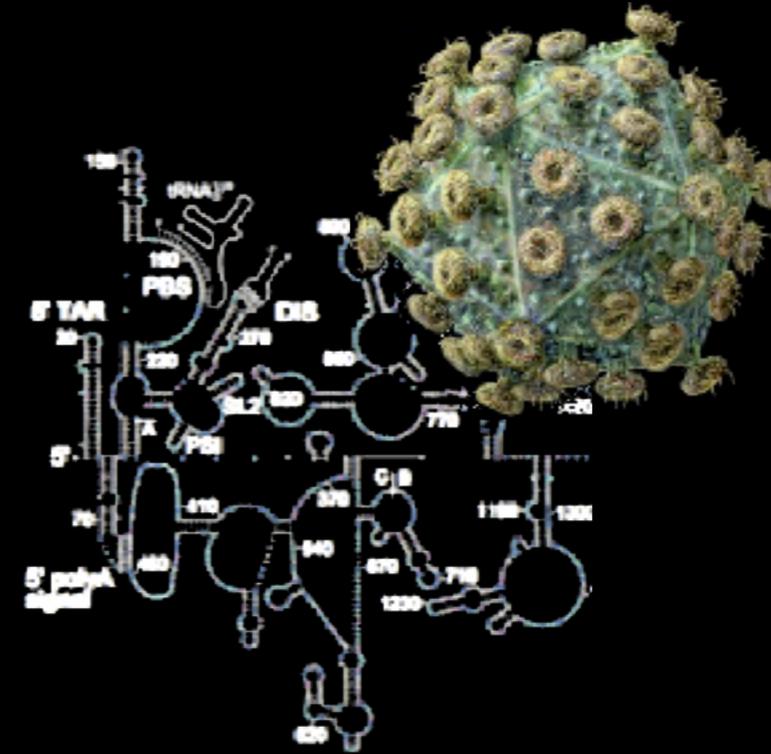
Stanford Biochemistry & Physics



RNA medicine



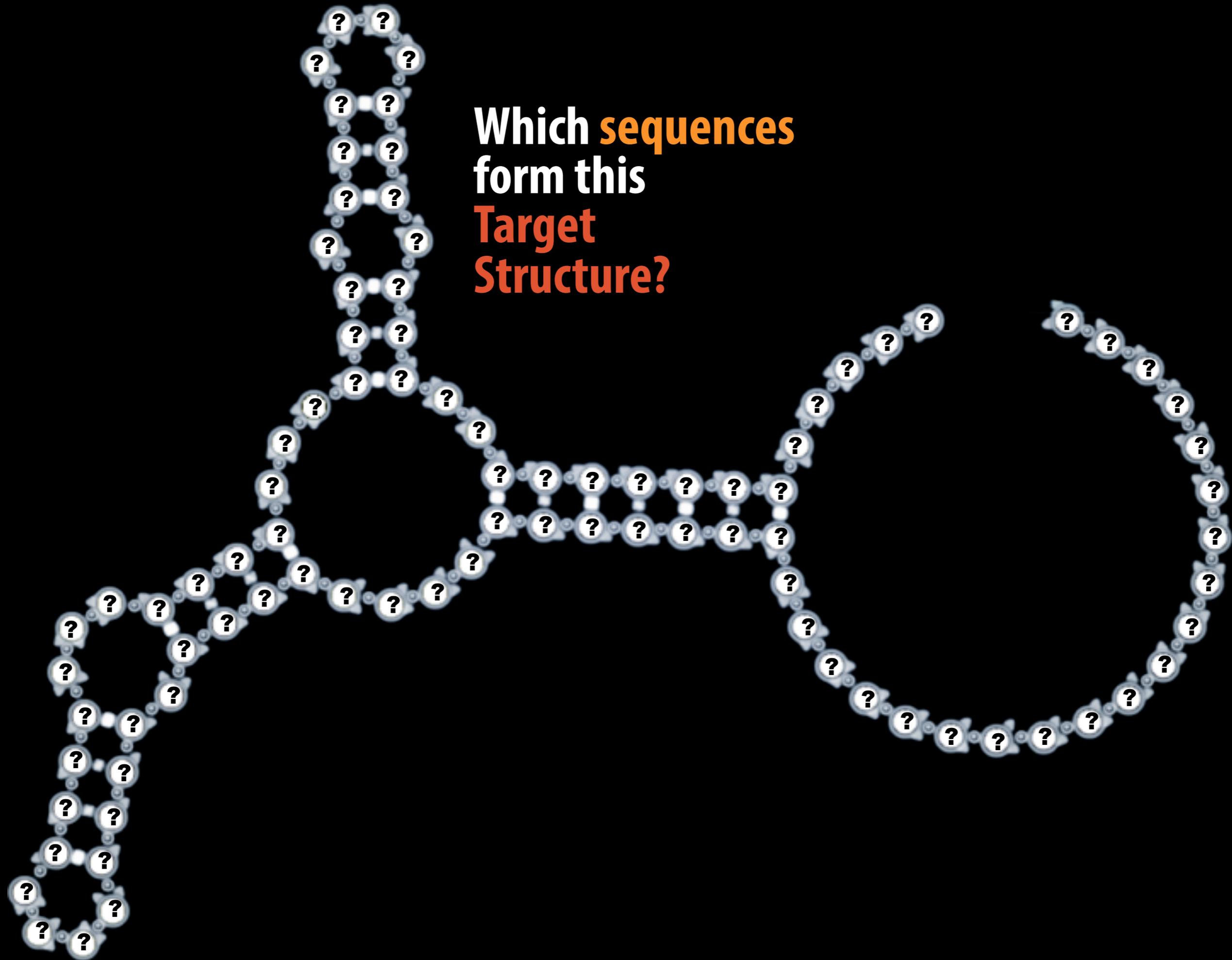
**Neurological and
Other Diseases**



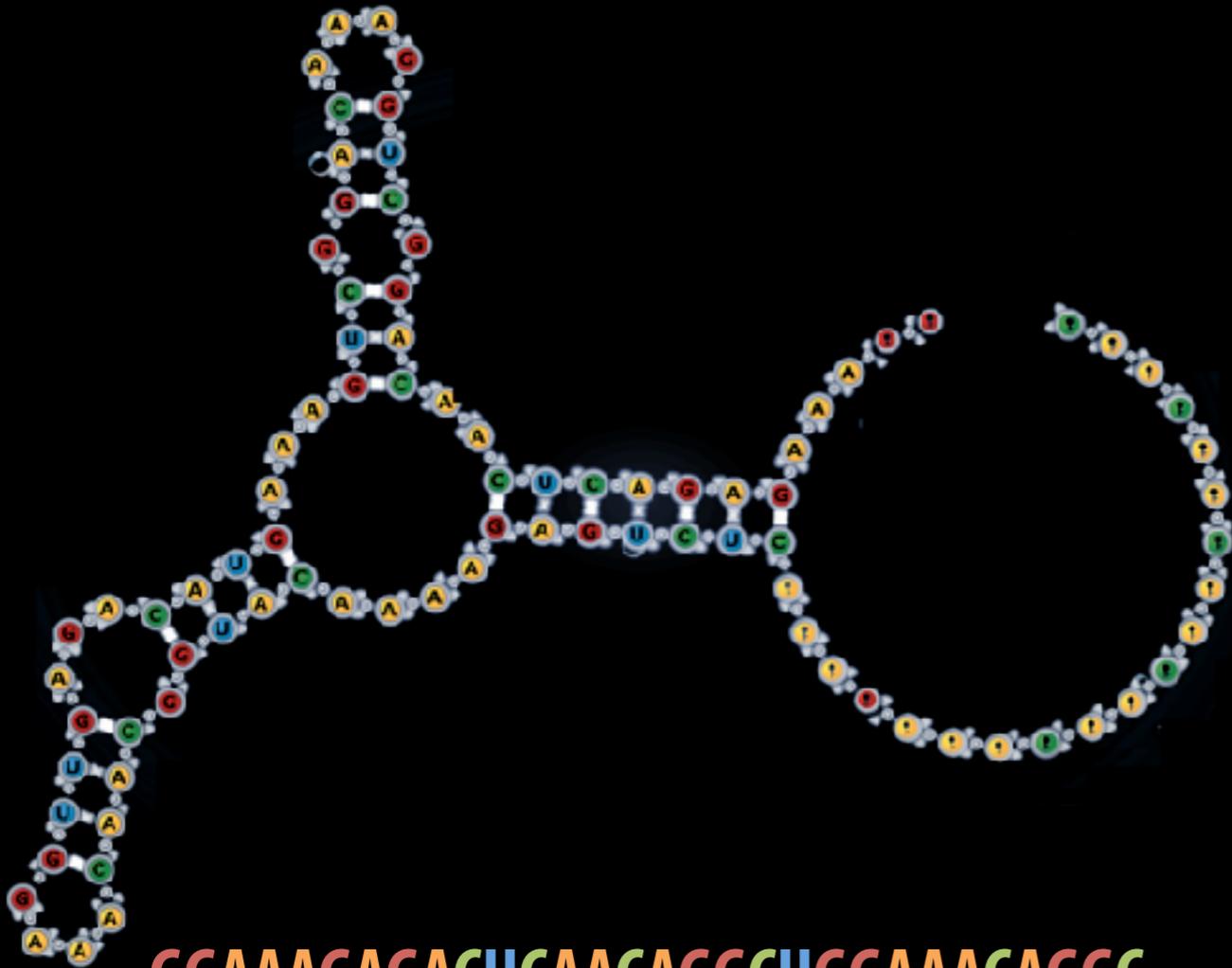
HIV-1 model from Weeks lab, UNC

**Retroviruses + other
pathogens**

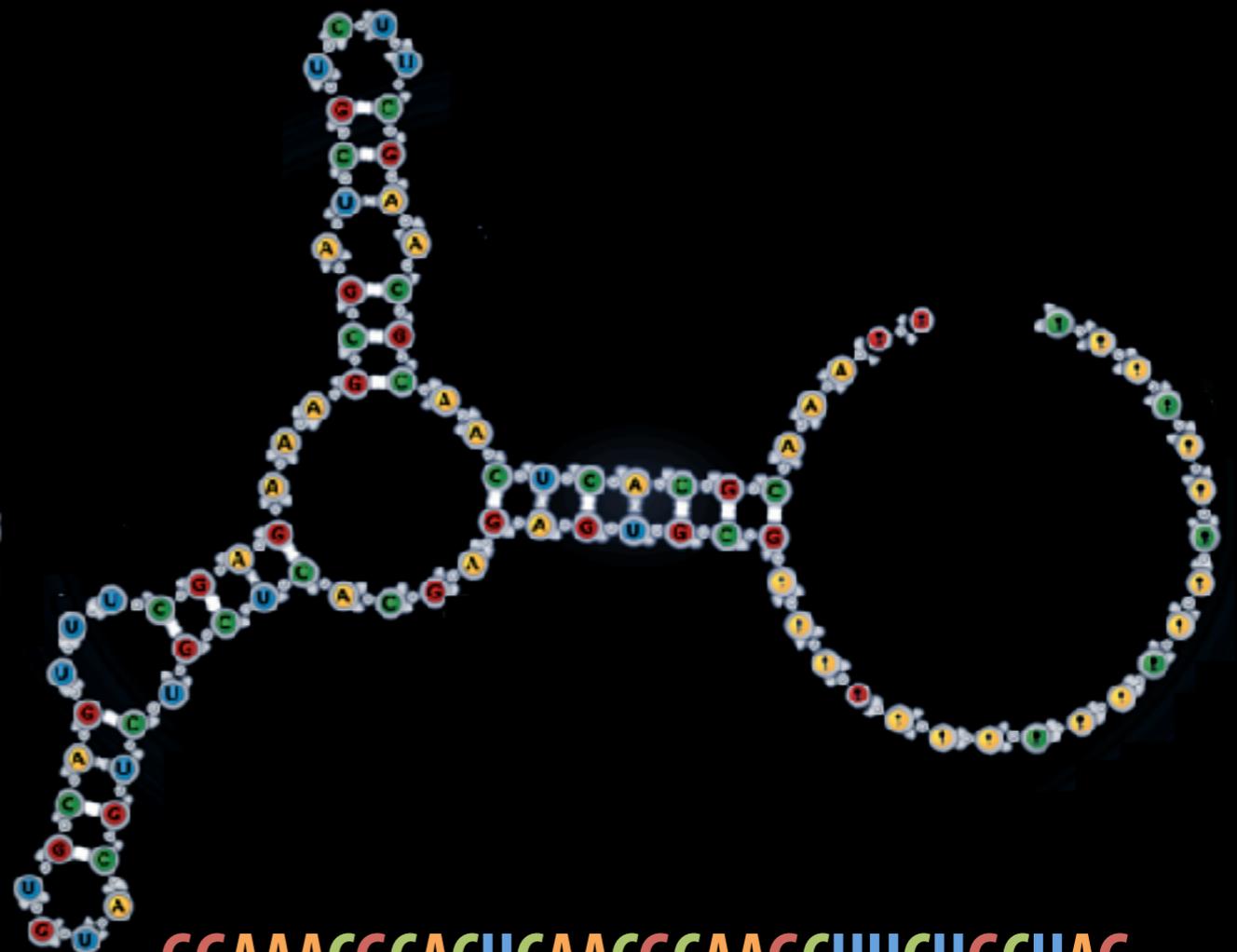
Which **sequences**
form this
Target
Structure?



Which answer is right?



GGAAAGAGACUCAACAGGCUGGAAACAGGC
UGAAAGUACAGAGUUGGAAACAACGGUACA
AAAGAGUCUCAAAGAAACAACAACAAC



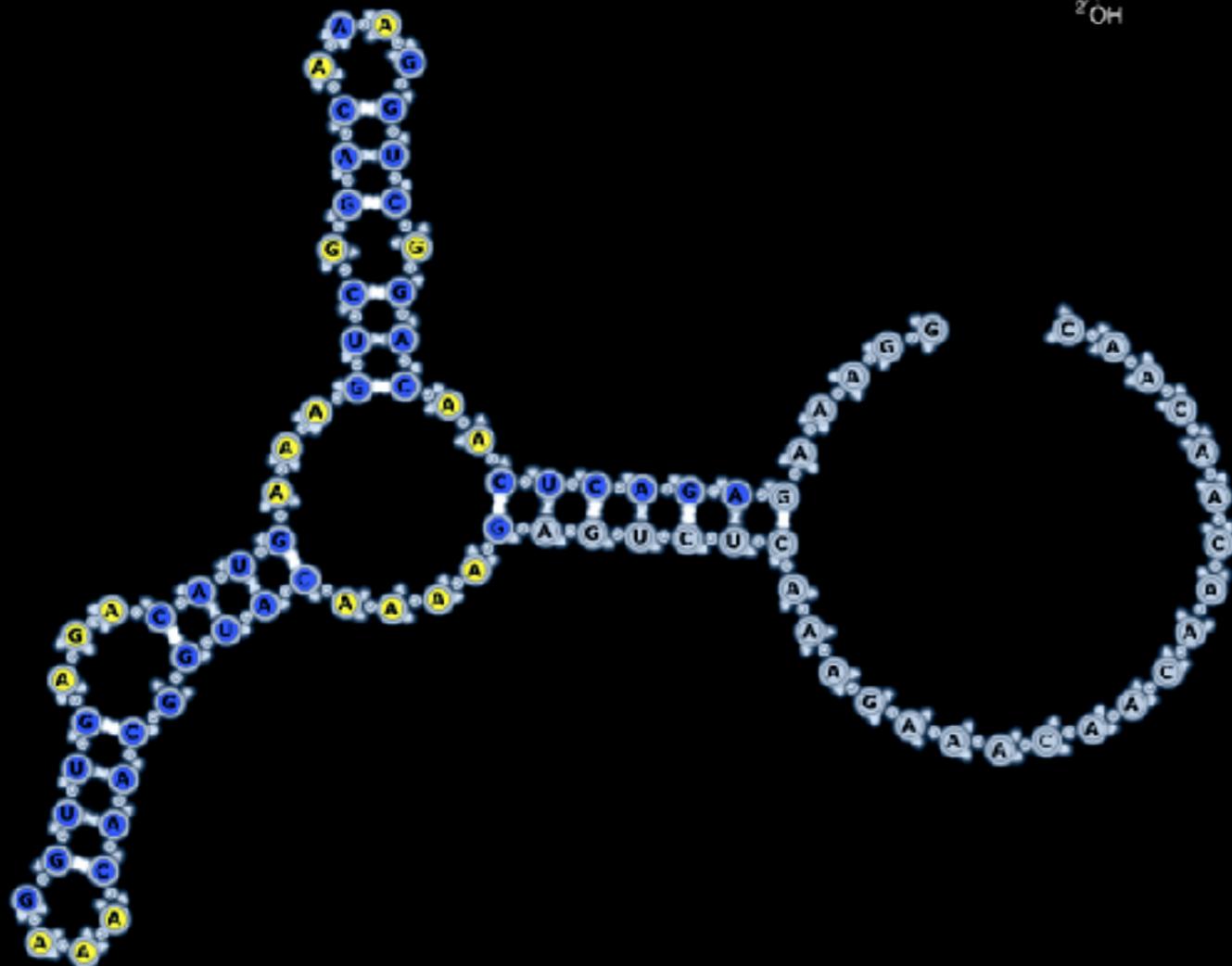
GGAAACGCACUCAACGCAAGCUUCUGCUAG
CGAAAGAGCUUUGACGUGUACGUCUGCUCA
CGAGAGU GCGAAAGAAACAACAACAAC

No expert or computer model can tell these designs apart.

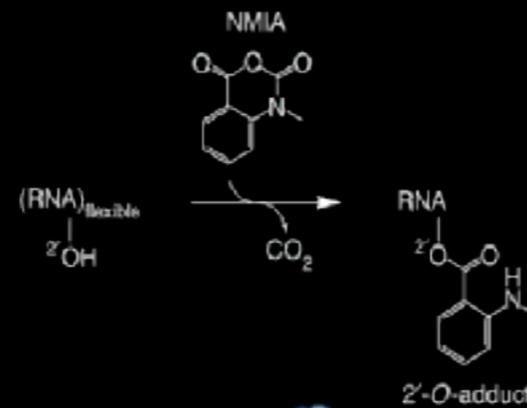
Which answer is right?

Nature Scores!

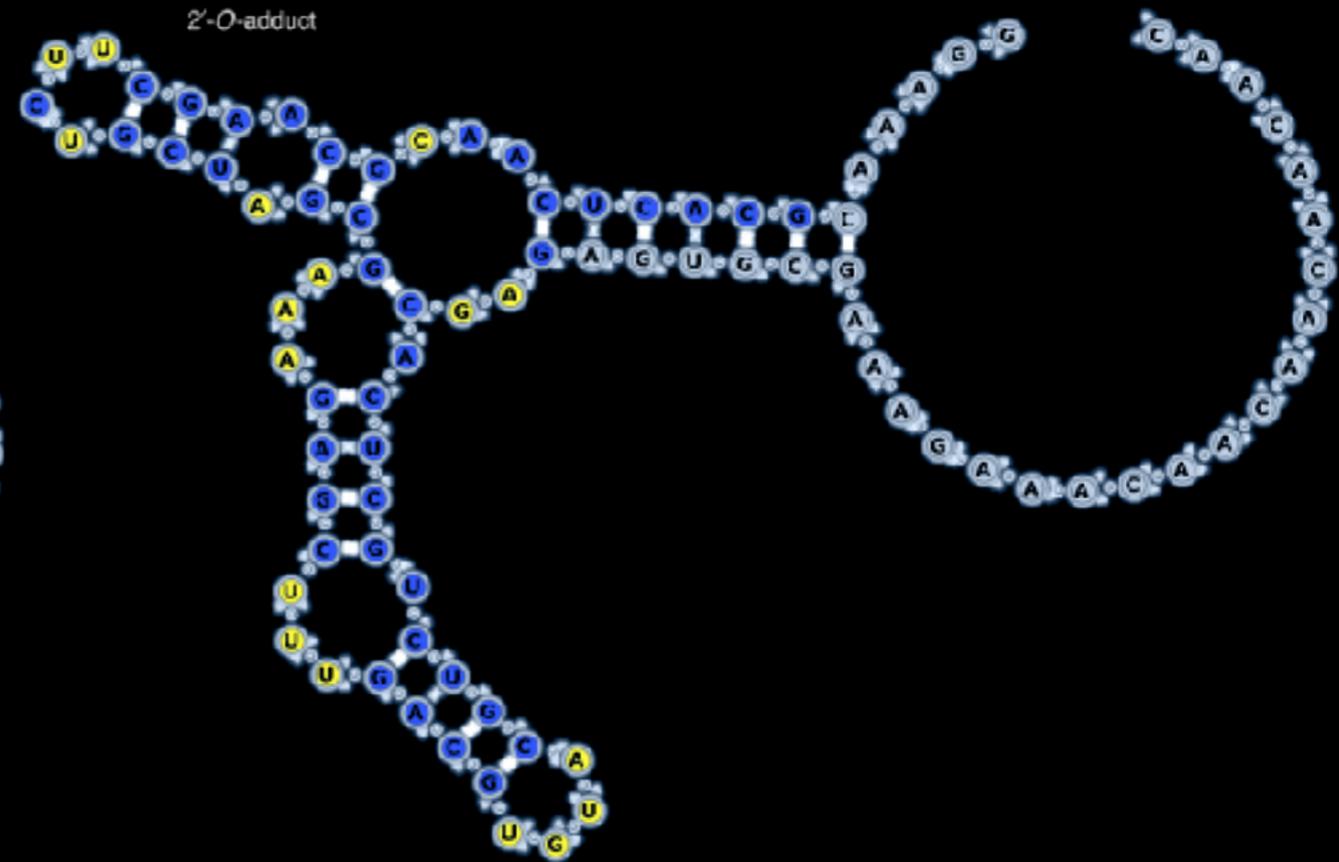
Correct



Taipan
by Penguin

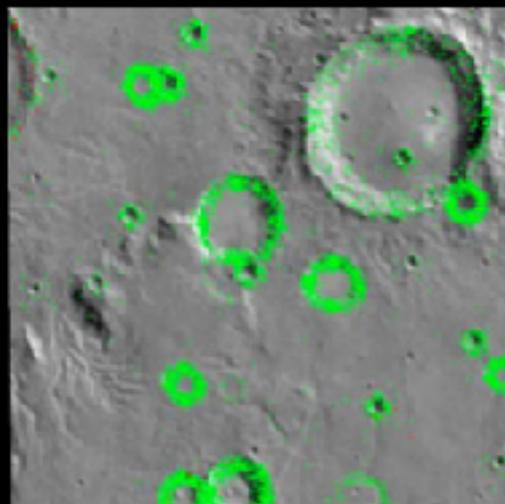


Incorrect



NUPACK 08
by NUPACK

Citizen Science



NASA ClickWorkers

2000

Image Analysis



Zooniverse Projects

2007

**Image and
Data Analysis**



Foldit

2008

**Computational
Bioscience**



EteRNA

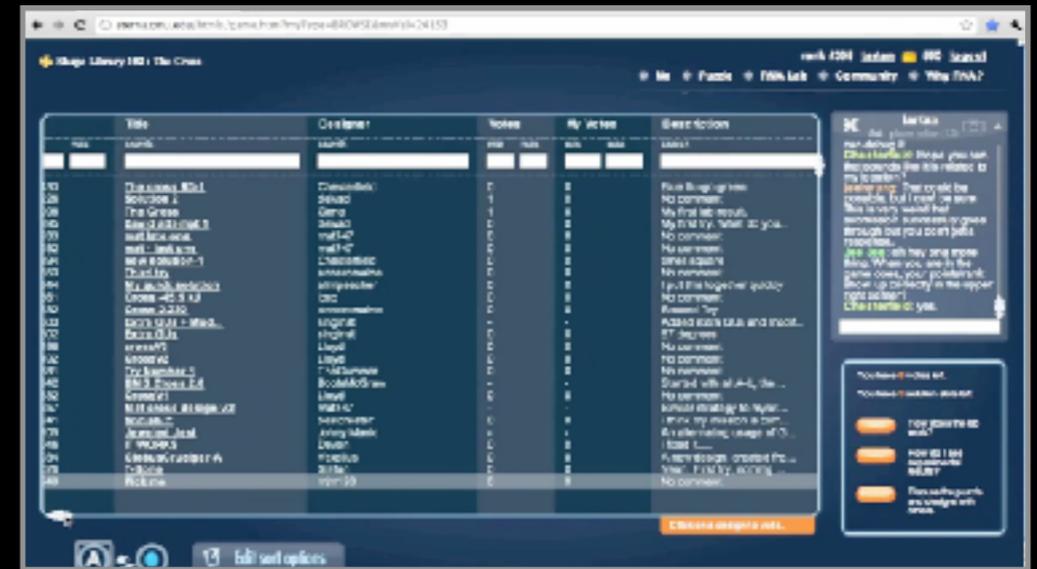
2011

**Experimental
Bioscience**

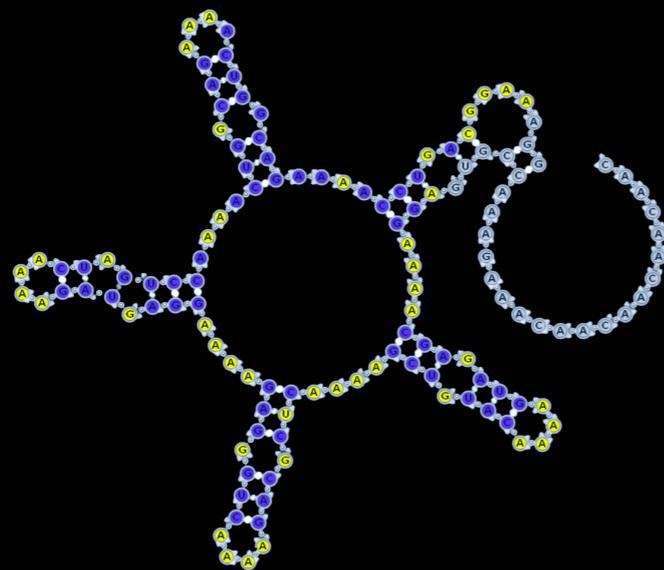
Played by Humans. Scored by Nature



Game Interface



Voting



Results



Synthesis

Played by Humans. Scored by Nature

**Crowdsourcing the
Scientific Method**

The Finger

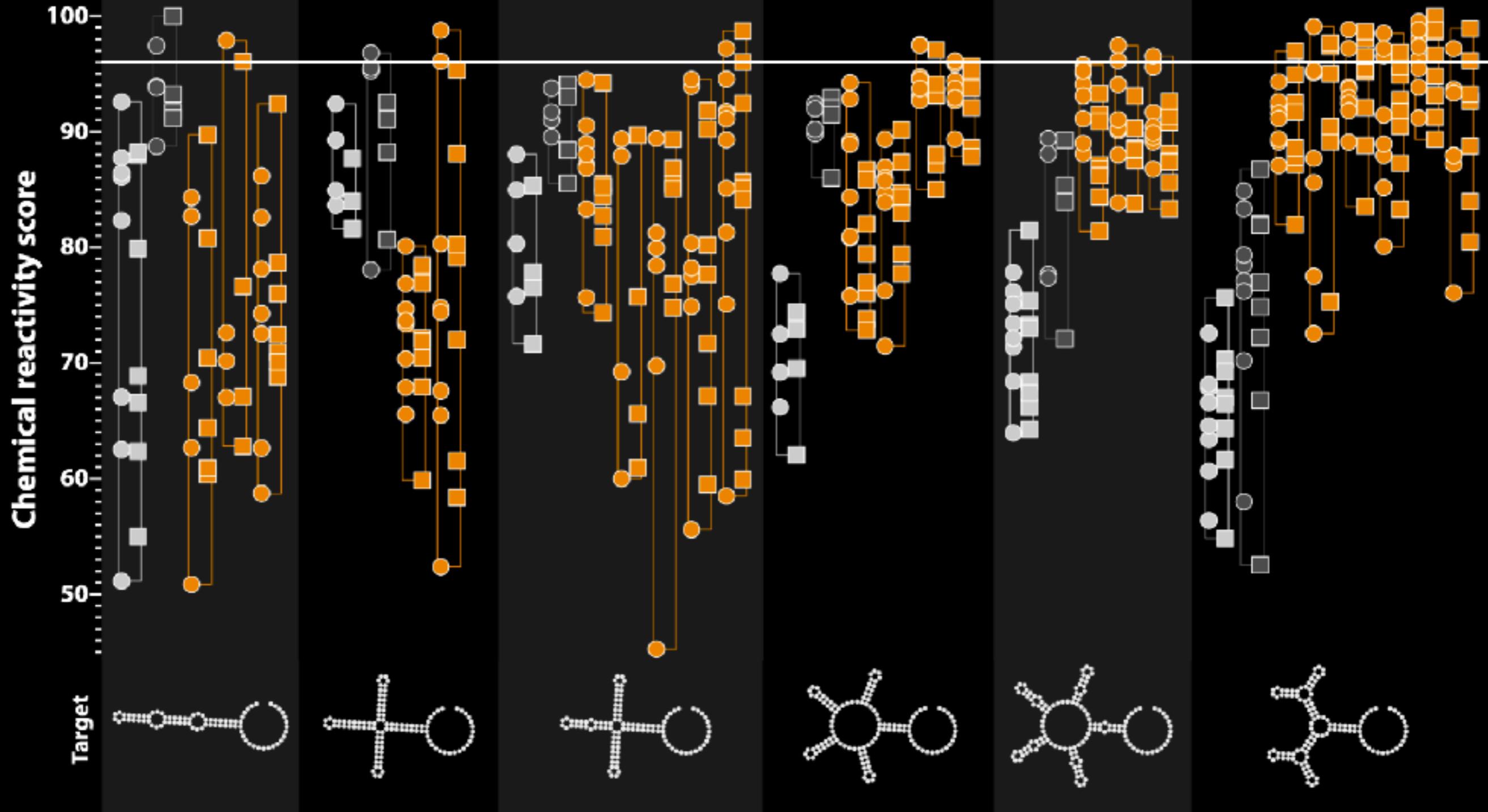
The Cross

Bulged Cross

The Star

Bulged Star

The Branches



Humans

Computers

The Finger

The Cross

Bulged Cross

The Star

Bulged Star

The Branches

Chemical reactivity score

How is this happening?

Humans

Computers

Target



Discovery of New Rules



All GC-pairs in the in multiloop junctions, have to turn in same direction. (Red nucleotide to the right and green nucleotide to the left.) Exception: the GC-pair connecting multiloop and neck, are allowed to turn in both directions, without being penalized.

~ Eli Fisker



Strategy Market Beta 2!

Tell us your strategy for picking successful designs in the lab and *help us build the first algorithm to score RNAs in nature!*

How well are EteRNA players doing?



Ensemble Strategy (coming soon!)

??
Ordering

Run!

All player strategies contribute to the **EteRNA Ensemble Strategy** which will be open to public. It is the *first algorithm* that predicts how well RNA sequences fold into the target shape in nature!

How to participate?

Post your Strategy

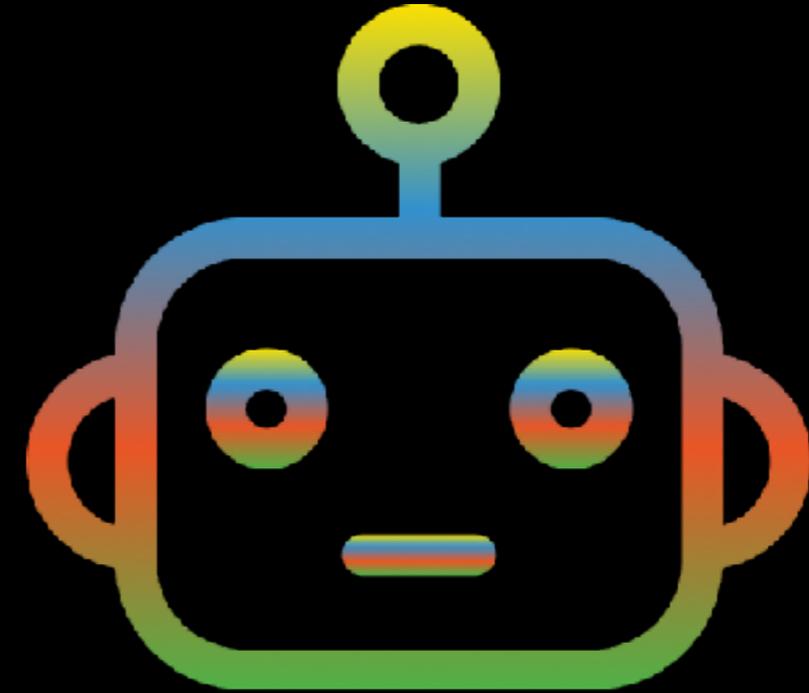
First, write your own strategy in the [forum](#) (See an [example post](#).) EteRNA developers will write an algorithm based on your post, test it on all synthesis results, and post results here! You can check out the test schedule for your strategy at [here](#)

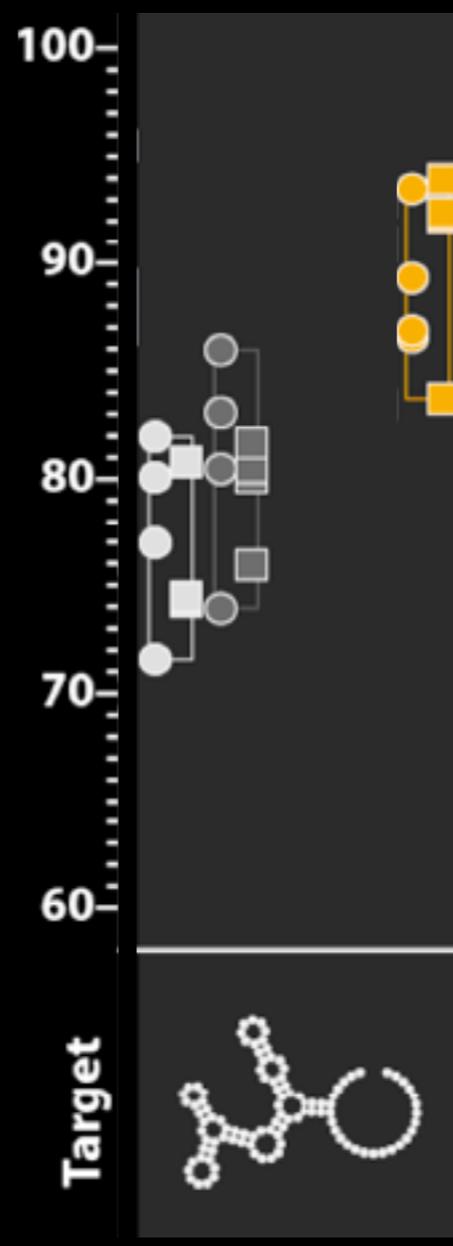
[Read more - what strategies do we want? What are all these numbers?](#)

Submitted Strategies

	aldo's Loops & Stacks	?? Contribution	0.443 Ordering	40 length	Run!
	Berex NZ's Berex Test	?? Contribution	0.431 Ordering	30 length	Run!
	xmbrst's Clean plot, stack caps, and safe GC	?? Contribution	0.381 Ordering	70 length	Run!
	delvad's delvad's strategy	?? Contribution	0.351 Ordering	60 length	Run!
	dejerpha's A basic test	?? Contribution	0.347 Ordering	20 length	Run!
	pengulan's Clean Dot Plot	?? Contribution	0.327 Ordering	10 length	Run!

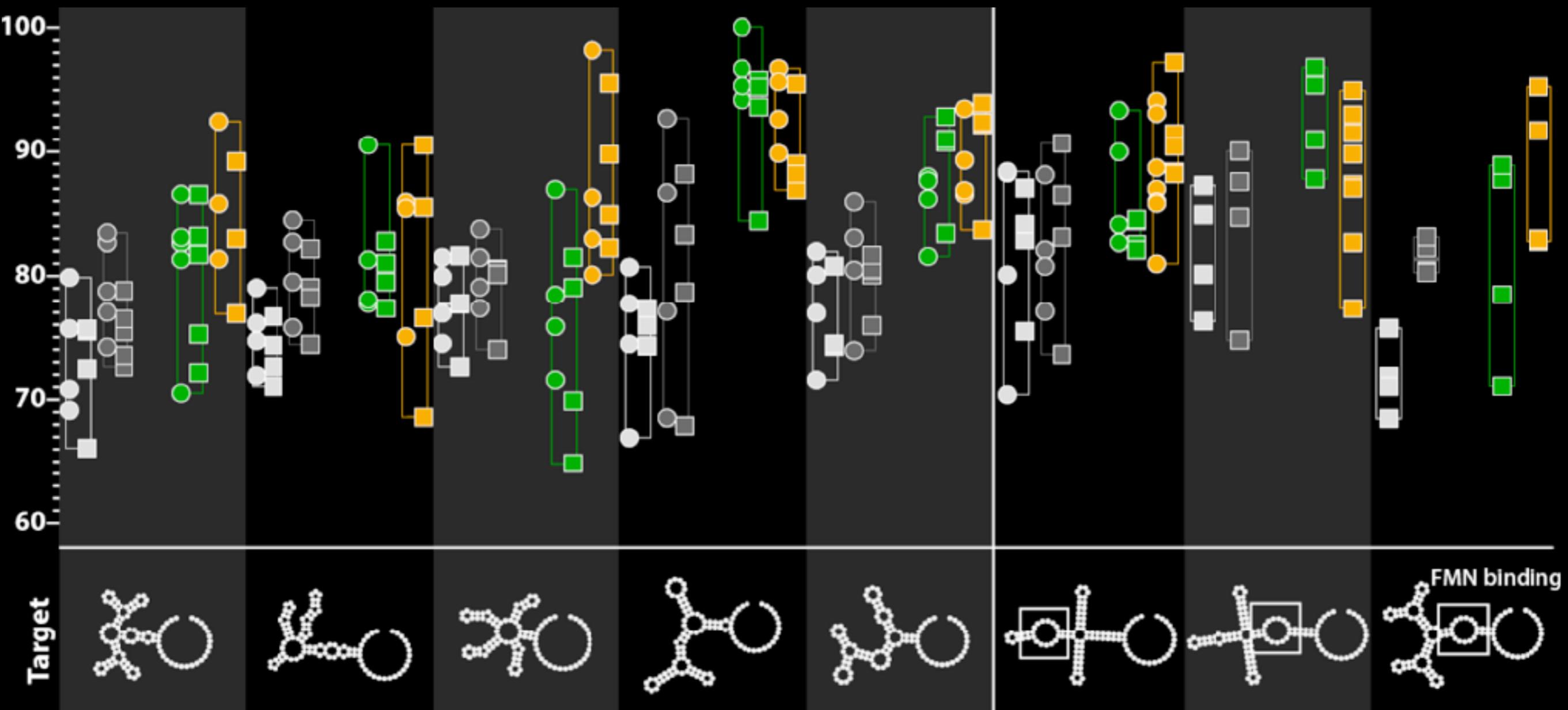
EteRNAbot





Humans

Computers



Humans

EternaBot

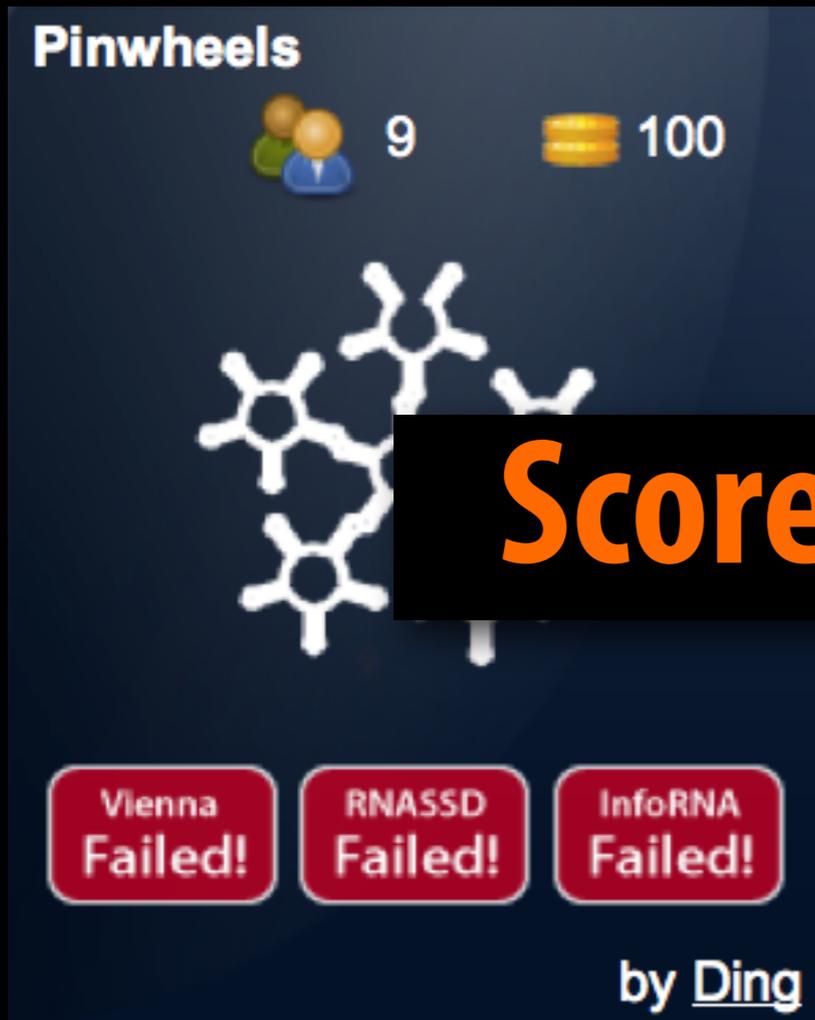
Computers

Iroppy: I like the idea of shapes and constraints that help us discover where the model does not work



Pinwheels

9 100



Score: ??

Vienna Failed! RNASSD Failed! InfoRNA Failed!

by Ding



eINando888

84,142 Registered Players

5,756 Lab Players

1000 Designs / round

32 Syntheses / round

Print

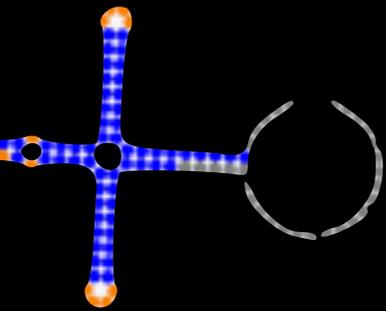


**Every design gets
synthesized**

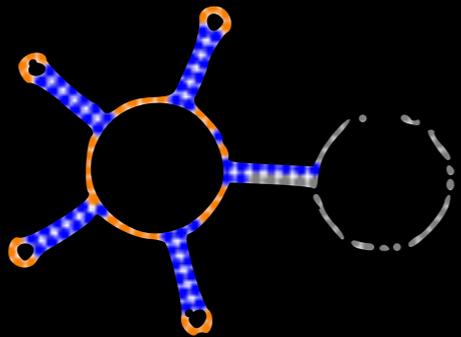
Probe



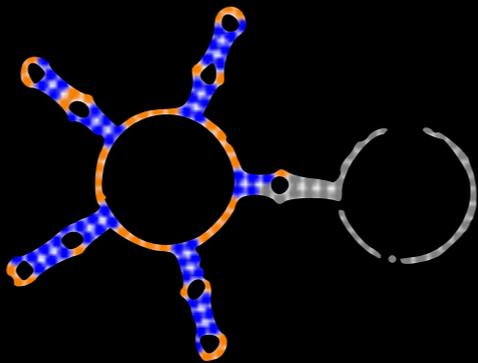
92



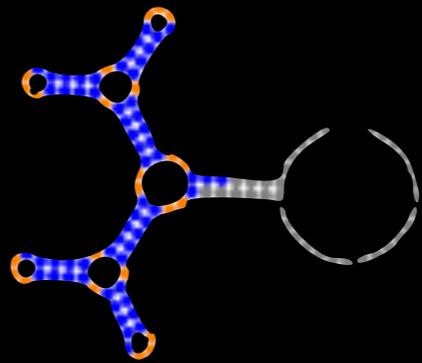
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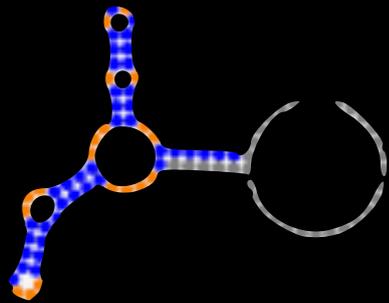
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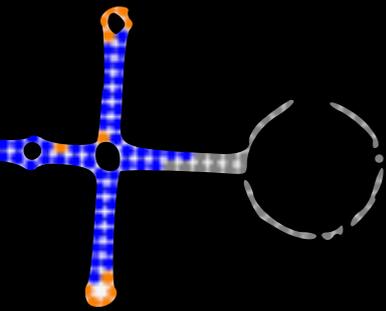
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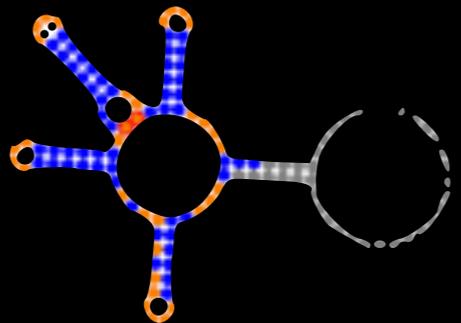
100



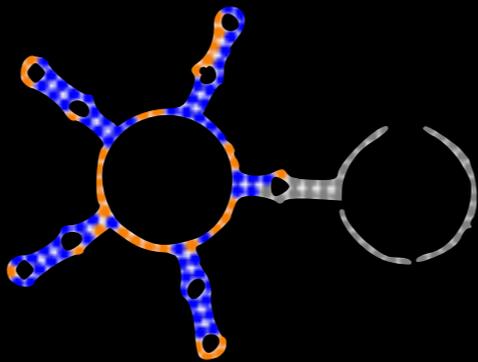
94



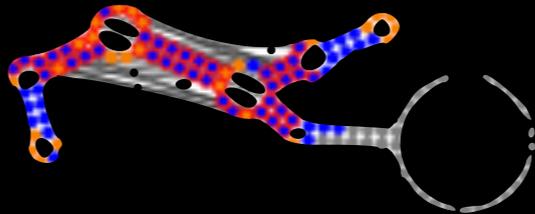
95



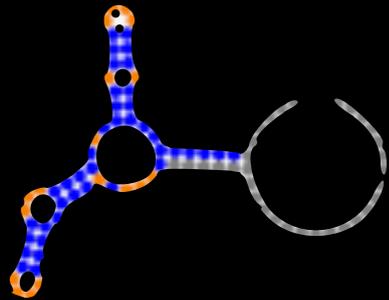
96



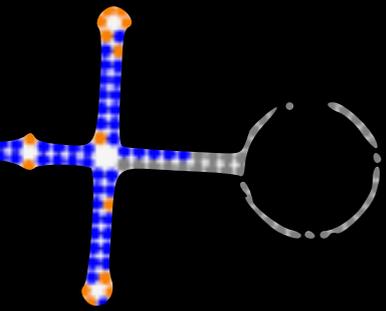
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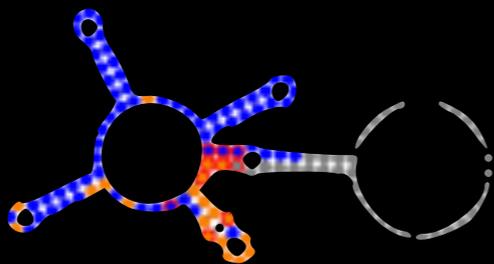
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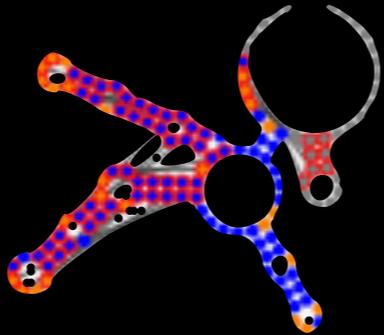
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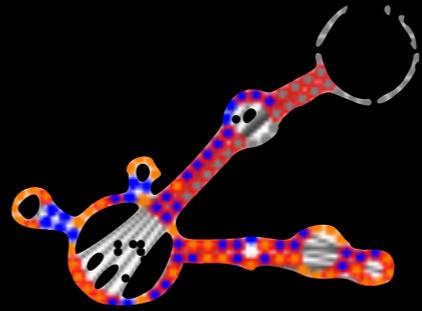
95



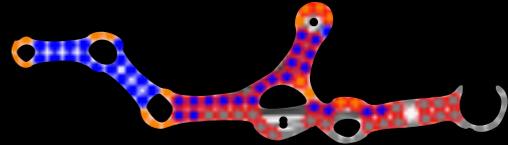
96



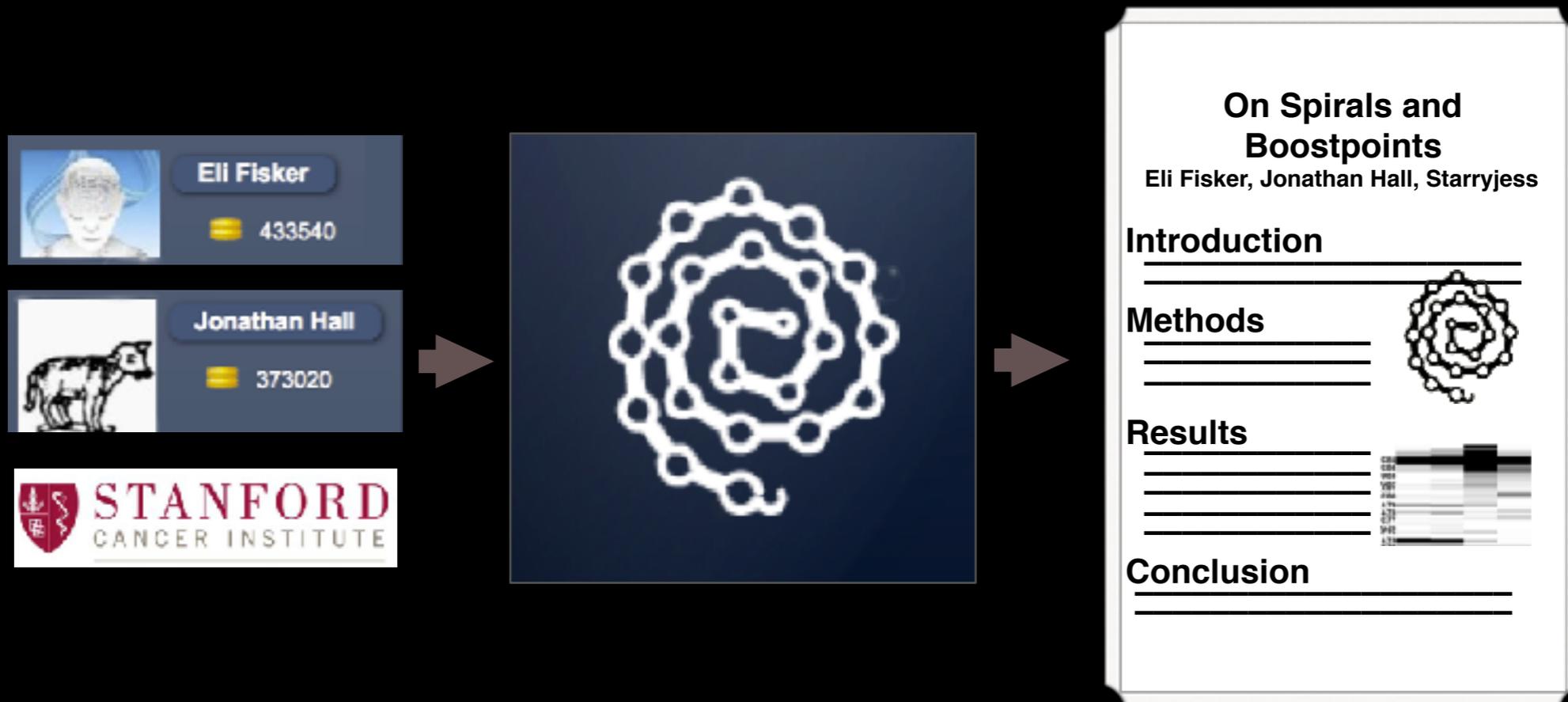
96



97



A new gameplay



**Complex
Hypothesis**

**Massively Parallel
Experiments**

Tiny Paper

Cloud Lab has synthesized **13613** sequences in **338** projects.

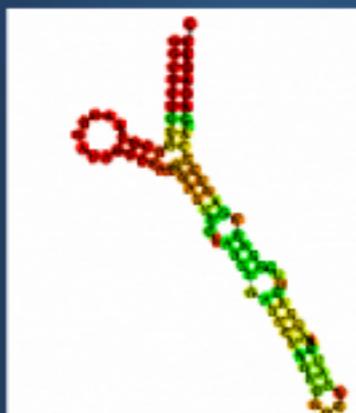
sort by [Puzzle post date](#) [Synthesis slot](#)

FEATURED

Alternative structure space for human tRNAs [\[Waiting for synthesis results\]](#)

featured by [University of California, Santa Cruz](#)

by [tmjlowe](#) on 17 May, 2014



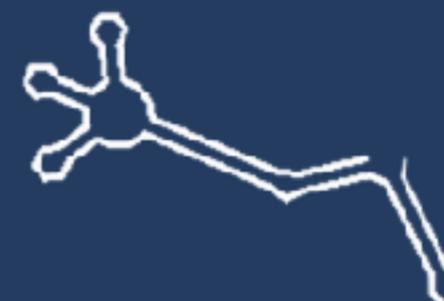
We wish to test propensity for cloverleaf versus alternative classes of structures for natural human tRNA variants among Arg, Met/Met, and Leu tRNAs.

[Review results](#)

[Lab Info](#)

Frog leg - Electric

Twin puzzle experiment - part 2



I wish to test how adjacent stacks in a multiloop of a lab design. I think designs with adjacent stacks are harder to solve than designs with multiple stacks. I have made two twin designs. The question, then, are how the stems are spaced in the multiloop join.

No animals were harmed in the experiment.

Winner (4 out of 4)

User	Name	Puzzle Title	Solution Title
	trycon93	Frog leg - Electric	TEST 2
	janetmason	Frog leg - Electric	Another frog leg

ZigZag and ZagZig

by [jandersonlee](#) on 26 Sep, 2013



Two tetraloop arms with Zig-Zags (one left leaning, one right leaning) branch off a relatively tame multiloop. Absent of unforeseen 3D interactions it should be a relatively simple template in which to try out some of the Zig Zag catalog in-vitro. No constraints in this round.

Winner (10 out of 10)

User	Name	Puzzle Title	Solution Title	Score
	Bround	ZigZag and ZagZig	Zig to the Zag - Bround - Lab 'ZigZag and ZagZig' R1 - Sub 1 (G-C light)	96
	trycon93	ZigZag and ZagZig	TEST 1	96

Modeled Impossible: 1 Nucleotide Bulge/Triloop Lonely base pair V1

by [Bround](#) on 10 Oct, 2013



In this lab, the goal is to stabilize the impossible secondary structure motif, of a bulge adjacent a triloop. Both triloop/bulge combinations are present here, and 60 designs will be chosen for synthesis. An identical project will be created in addition to this one, with a constrained sequence for the bulge. I hope to see interesting designs and sequences from players!

Winner (10 out of 15)

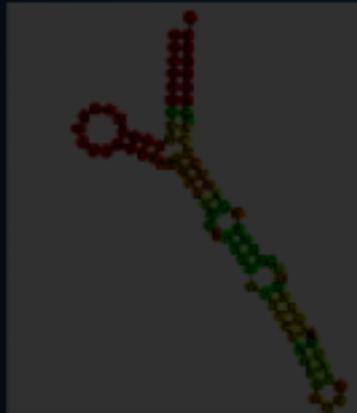
User	Name	Puzzle Title	Solution Title
	Bround	Modeled Impossible: 1 Nucleotide Bulge/Triloop Lonely base pair V1	Ledible - Bround - Lab 'Modeled Impossible: 1 Nucleotide Bulge/Triloop Lonely base pair V1 R1 - Sub 3 (GAGCACCC + OCCACGUG)
	Ormei	Modeled Impossible: 1 Nucleotide Bulge/Triloop Lonely base pair V1	Mod of Bround's Sub 1

Cloud Lab has synthesized **41855** sequences in **379** projects.

FEATURED

Alternative structure space for human tRNAs [Waiting for synthesis results]
featured by University of California, Santa Cruz

by [lmjlowe](#) on 17 May, 2014



We wish to test propensity for cloverleaf versus alternative classes of structures for natural human tRNA variants among Arg, Met/Met, and Leu tRNAs.

Review

Google scholar

RNA spiral



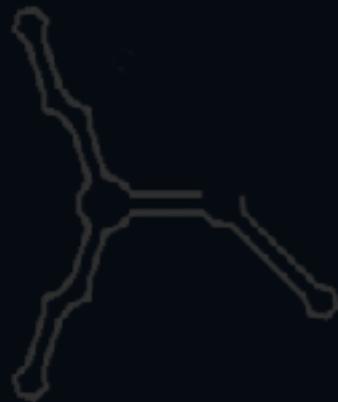
[On RNA spirals and boostpoints](#)

E Fisker, [starryjess](#), B. Townshend - eternagame

... The arrow indicates the presumed direction of RNA exit. The spiral model obtained

Scientific Literature

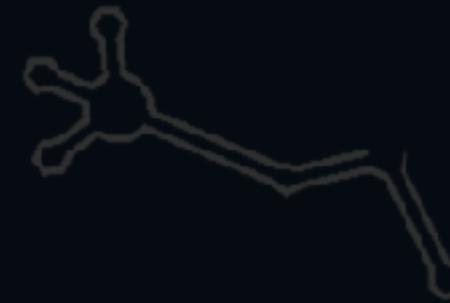
ZigZag and ZagZig



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Winner (10 out of 10)

User	Name	Puzzle Title	Solution Title	Score
	Bround	ZigZag and ZagZig	Zig to the Zag - Bround - Lab ZigZag and ZagZig' R1 - Sub 1 (G-C light)	96
	tryoon93	ZigZag and ZagZig	TEST 1	96



I wish to test how adjacent stacks in a multiloop of a lab design. I think designs with adjacent stacks are harder to solve than designs with multiple stacks. I have made two twin designs. I think the most interesting ones, are how the stems are spaced in the multiloop join.

No animals were harmed in the experiment

Winner (4 out of 4)

User	Name	Puzzle Title	Solution Title
	tryoon93	Frog leg - Electric	TEST 2
	janelmason	Frog leg - Electric	Another frog leg

Modeled Impossible: 1 Nucleotide Bulge/Triloop Lonely base pair V1

by Bround

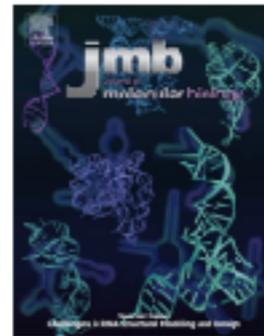


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Winner (10 out of 15)

User	Name	Puzzle Title	Solution Title
	Bround	Modeled Impossible: 1 Nucleotide Bulge/Triloop Lonely base pair V1	Ledble - Bround - Lab Modeled Impossible: 1 Nucleotide Bulge/Triloop Lonely base pair V1 R1 - Sub 3 (GAGCACCC + OCCACGUG)
	Ornel	Modeled Impossible: 1 Nucleotide Bulge/Triloop Lonely base pair V1	Mod of Bround's Sub 1

Cloud Lab has synthesized **41855** sequences in **379** projects.



Principles for Predicting RNA Secondary Structure Design Difficulty

Jeff Anderson-Lee^{1,†}, Eli Fisker^{1,†}, Vineet Kosaraju^{1,2,†}, Michelle Wu^{1,3,†}, Justin Kong^{1,4}, Jeehyung Lee^{1,4}, Minjae Lee^{1,4}, Mathew Zada¹, Adrien Eterna

1 - Eterna
2 - Depart
3 - Program
4 - Depart
5 - Roboti
6 - Depart

Evidence of an Unusual Poly(A) RNA Structure Detected by High-throughput Chemical Mapping

Roger Wellington-Oguri,* Eli Fisker, Mathew Zada, Michelle Wiley, Eterna Players

Eterna Massive Open Laboratory.

Supporting Information Placeholder

bioRxiv preprint first posted online Jun. 14, 2018; doi: <http://dx.doi.org/10.1101/345667>. The copyright holder for this preprint (which was not peer-reviewed) is the author/funder. All rights reserved. No reuse allowed without permission.

An unexpectedly effective Monte Carlo technique for the RNA inverse folding problem

1
2
3
4 Fernando Portela^{1,2}
5

6 ¹ Citizen scientist, member of the Eterna Massive Open Laboratory

7 ² Department of Biochemistry, School of Medicine, Stanford University, CA 94305

8 * Corresponding author. Email: nando8888@gmail.com
9

Correspo
rhiju@stan
http://dx.d
Edited by

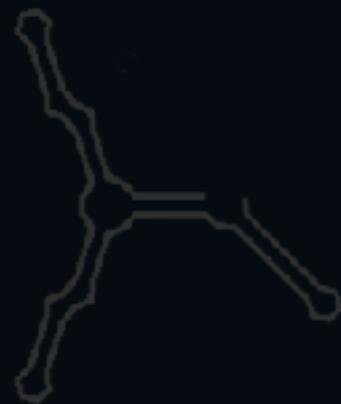
Abstract

Designing systems about wh time and into spec secondar automate Subsequ confirmed the hypothe sequence length, mean on these results, we hav that span a large range improving computational single RNA structures, a

ABSTRACT
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the Eterna M
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substitution.

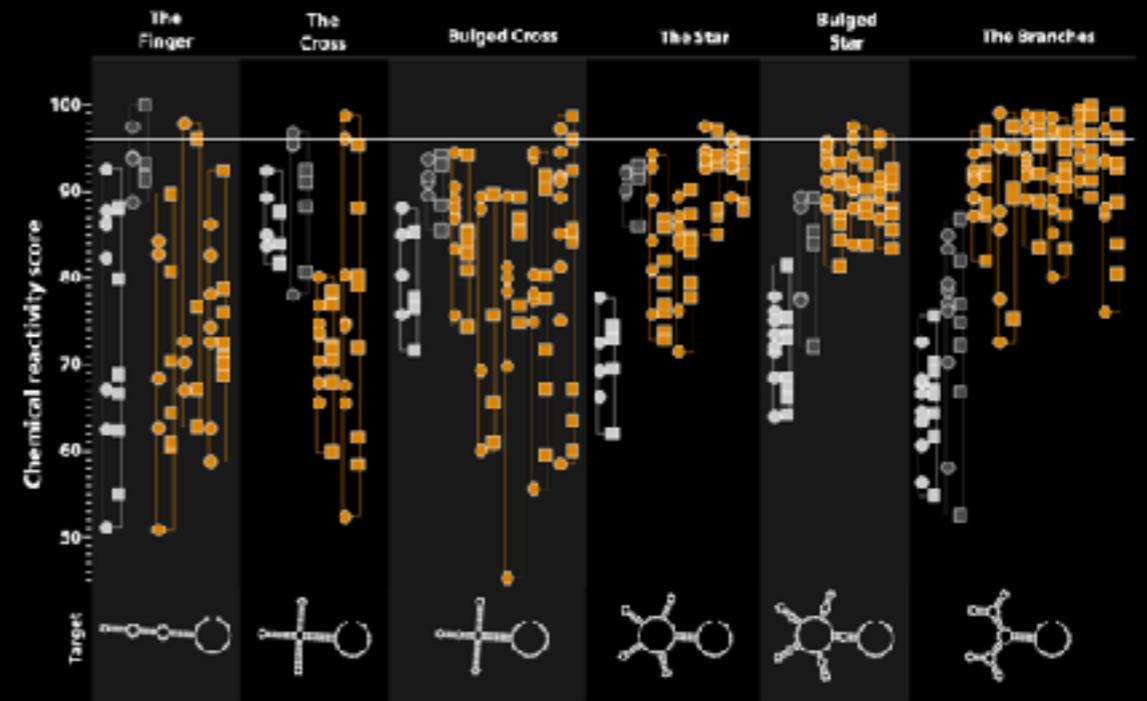
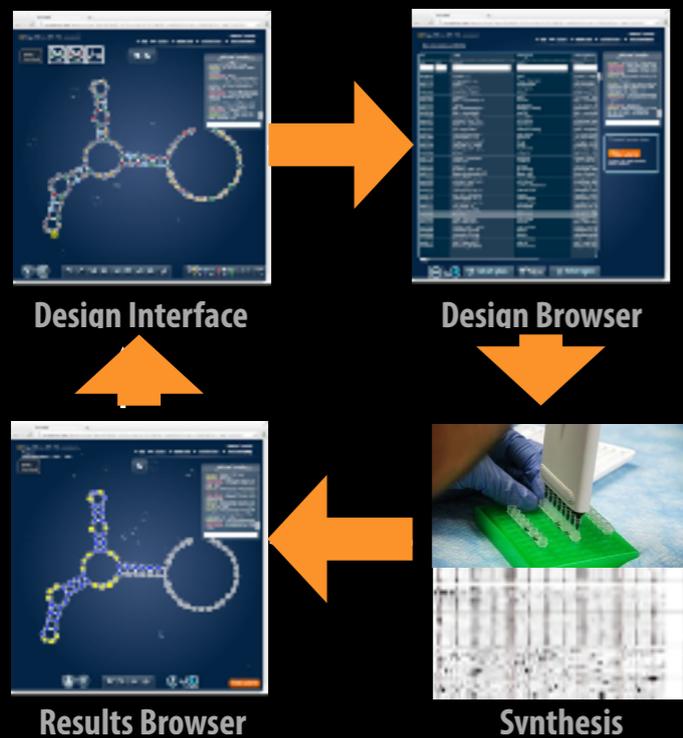
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ZigZag and ZagZig



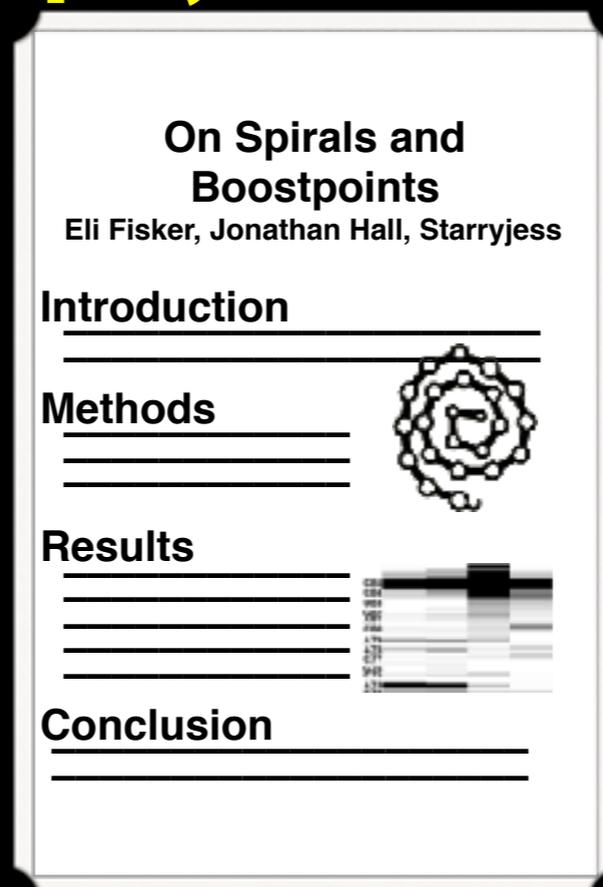
Winner (10 out of 10)

User	Name	Puzzle Title
	Broud	ZigZag and ZagZig
	tryoon99	ZigZag and ZagZig



Experiments in game play

Predictive power: real-world RNA design



Players as scientific authors

Silicon Valley: we need you!



<https://eternagame.org>

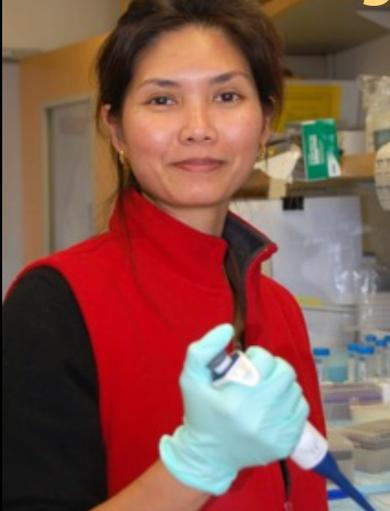
Eterna

150,000
registered
players



The biggest
games have
>2,500,000,000
downloads

Ann Kladwang



Johan Andreasson



**Ben Keep
Sharif Ezzat
Caleb Geniesse**

**Prof. Purvesh Khatri
Prof. Will Greenleaf**



National Institutes of Health

BILL & MELINDA GATES foundation



Adrien Treuille (Carnegie Mellon)

Jee Lee



Eli Fisker

439080



starryjess

432850



Joshua Weitzman

398906



mat747

446768



madde

333798



chaendryn

373160



Bacteriofago

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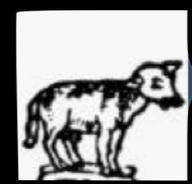
Chesterfield

394080



Duende

332750



Jonathan Hall

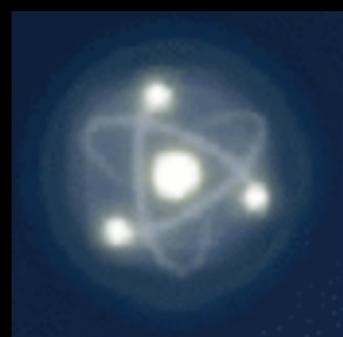
373020



FOLD



El Nando888



jnicol



omei



LFP6



tncannon

327430



clollin

398536



Engineseer

280690

