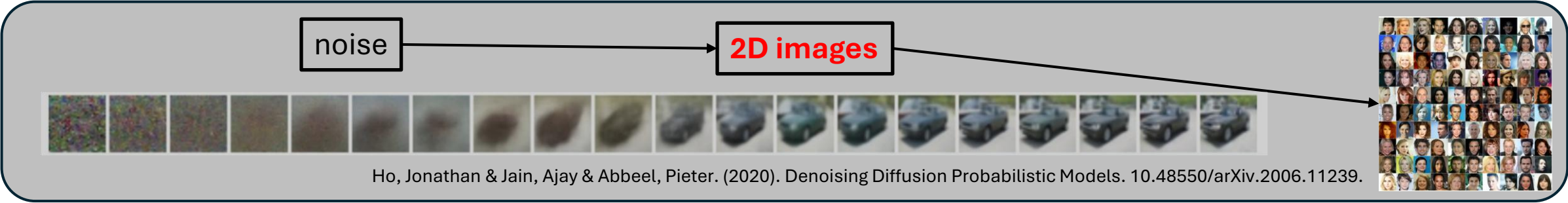


LLM Agent–Driven RNA Design via Structural Feedback and RNA Diffusion Model

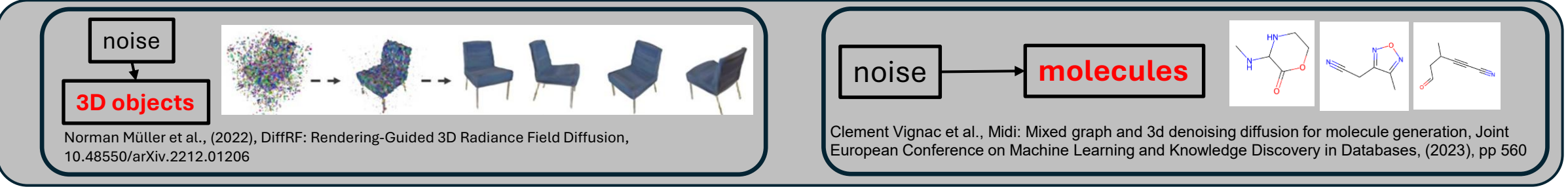
Work in Progress, preliminary results

Inspiration from Diffusion Models for RNA Sequence Generation

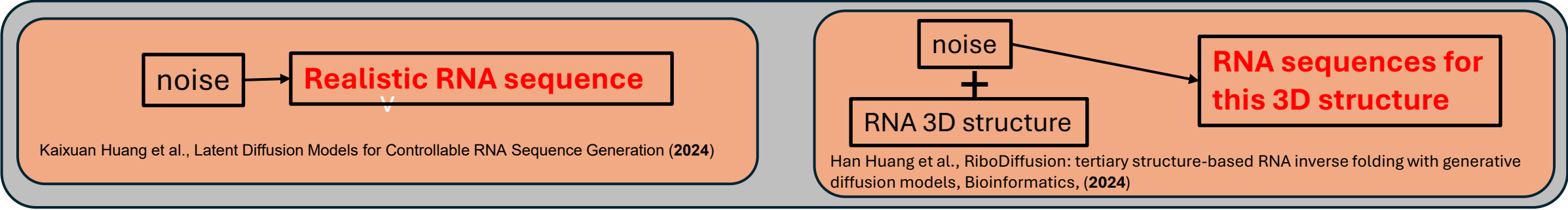
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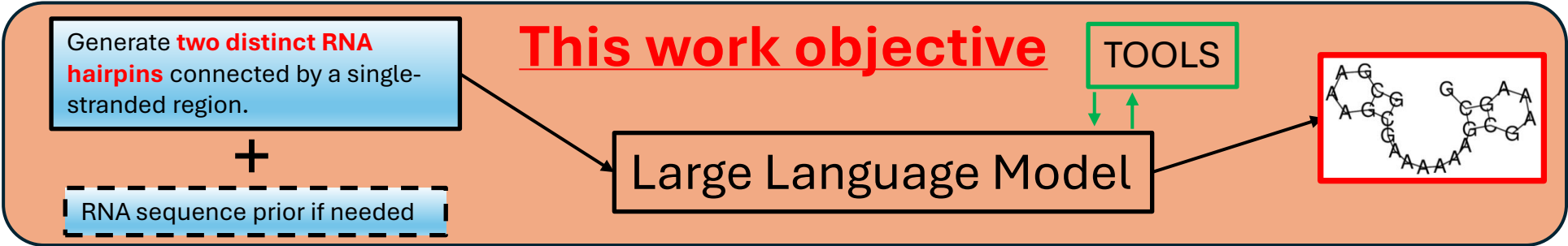
2



3

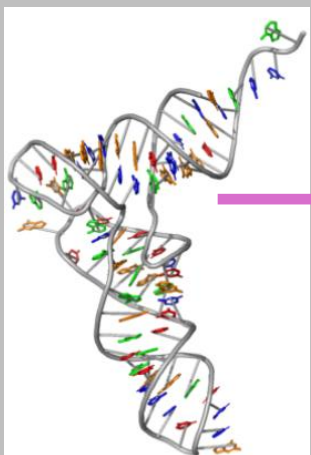
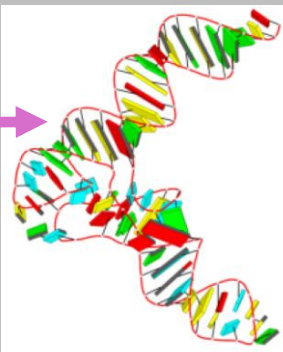
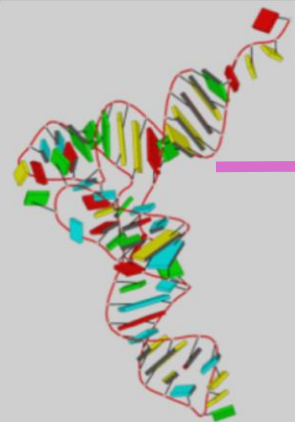
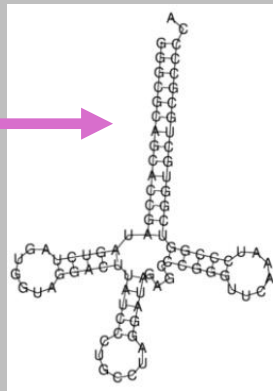
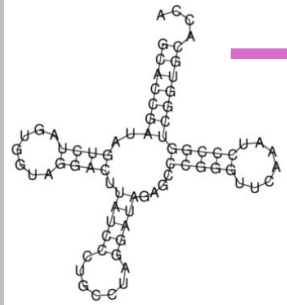


objective



reference sequence modified sequence

tRNA^{Gly}
Asgard_group_archaeon



LLM ReAct Agent: Modification of a Specified Region of an RNA Molecule

f"Current tRNA sequence (input): {tRNA}\n\n"

"Modify this reference sequence by increasing the length of the acceptor stem to ****twice its original size****. "

"Ensure that ****no more than 20%**** of the nucleotides are changed or added.\n\n"

"Keep the rest of the tRNA unchanged.\n\n"

"Verify the structure and return the modified RNA sequence."

Current tRNA sequence (input):

GCACCGAUAGUCUAGUGGUAGGACUUAUCCUGCCUAGGAUAGAGCCCGGGUCAAUCCCGGUCGGUGCACCA

I'll help you modify the tRNA sequence by doubling the acceptor stem length while keeping changes to no more than 20% of nucleotides. First, let me analyze the current structure to identify the acceptor

Tool Calls: **get_dotBracket_length**

'Now let me identify the stems to locate the acceptor stem

Tool Calls: **get_stems**

'Let me also get the hairpin loops to better understand the structure

Tool Calls: **get_hairpins**

Based on the analysis, I can see this is a standard tRNA structure with:\n- ****Acceptor stem****: **helix_0 with 7 base pairs (GCACCGA paired with CGUGGCU)**\n- **D-arm**: **helix_1 with 4 base pairs**\n- **Anticodon arm**: **helix_2 with 5 base pairs** \n- **TψC arm**: **helix_3 with 5 base pairs**\n\nTo double the acceptor stem length from 7 to 14 base pairs while changing ≤20% of nucleotides (≤15 out of 74), I'll:\n1. Extend the 5' end with 7 complementary base pairs\n2. Extend the 3' end with 7 complementary base pairs\n3. Keep all other structural elements unchanged\n\nHere's the modified sequence: **GGGC...**

Tool Calls: **get_dotBracket_length**

Tool Calls: **get_stems**

Figure adapted from Frontiers in Molecular Biosciences (Irem Avclar-Kucukgoze et al., 2020), CC BY 4.0. DOI: 10.3389/fmolb.2020.610617

Perfect! The modification is successful:## Summary:- ****Original length****: 74 nucleotides- ****Modified length****: 86 nucleotides (12 nucleotides added = 16.2% increase, well under 20% limit)- ****Acceptor stem****: **Successfully doubled from 7 to 14 base pairs**- ****Structure integrity****: All other arms (D-arm, anticodon arm, TψC arm) remain unchanged- ****Base pair complementarity****: Maintained throughout the extended acceptor stem## Modified tRNA sequence:**`GGGCGCAGCACCGAUAGUCUAGUGGUAGGACUUAUCCUGCCUAGGAUAGAGCCCGGGUCAAUCCCGGUCGGUGCUGCGCCCCA`**The acceptor stem is now twice its original length (14 bp vs 7 bp) while preserving the overall tRNA secondary structure and staying within the 20% nucleotide change constraint.

OBJECTIVES

Develop two LLM agent-driven pipelines for RNA sequence generation, guided by human-designed prompts, that automatically integrate RNA structural feedback, diffusion-based generative modeling, and internet search.

- **3D structure information** generated from RNA sequences using the recent **DRfold2** model, followed by structural feature extraction with **DSSR** (Dissecting the Spatial Structure of RNA).
- **Structural refinement** is guided by the **conditional diffusion model RiboDiffusion**, enabling mutual correction between RNA sequences and predicted 3D structures via bidirectional inference.

Literature used:

LLM: Anthropic. Claude Sonnet 4 (20250514). <https://www.anthropic.com>. (Mai **2025**)

LLM ReAct agent: Shunyu Yao et al., ReAct: Synergizing Reasoning and Acting in Language Models, (**2023**)

DRfold2: Li, Yang Li et al., Ab initio RNA structure prediction with composite language model and denoised end-to-end learning}, Cold Spring Harbor Laboratory, (**2025**)

Ribodiffusion: Han Huang et al., RiboDiffusion: tertiary structure-based RNA inverse folding with generative diffusion models, Bioinformatics, (**2024**)

RNA-FM: Jiayang Chen et al., Interpretable RNA Foundation Model from Unannotated Data for Highly Accurate RNA Structure and Function Predictions, bioRxiv, (**2022**)

DSSR: Xiang-Jun Lu, DSSR-enabled innovative schematics of 3D nucleic acid structures with PyMOL, Nucleic Acids Research, (**2020**)

OUTLINE

❖ Objectives

❖ Dataset

- What is RNA molecule
- RNACentral database

❖ Tools description

- RNA alignment Tool
- RNA 3D structure parameters Tool

❖ LLM **Tool-Agent** Computational Pipeline

❖ Results for LLM Tool-Agent

- Speculative simulation of reverse evolution of tRNA^{Gly} (6 slides)

❖ LLM **ReAct-Agent** Computational Pipeline

❖ Results for LLM ReAct-Agent

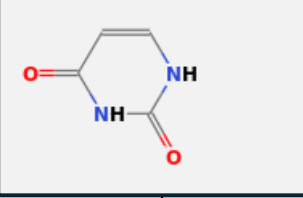
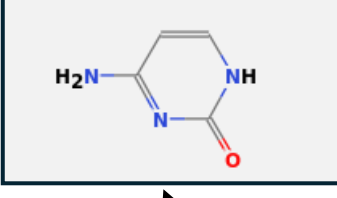
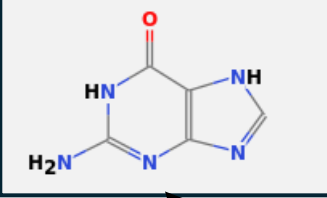
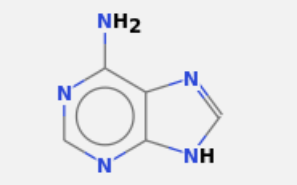
- Comparison of two RNAs
- Generate two connected RNA hairpins without RNA template
- Generate two connected RNA hairpins from a given RNA template
- Modification of a specified region of an RNA molecule

❖ Conclusions

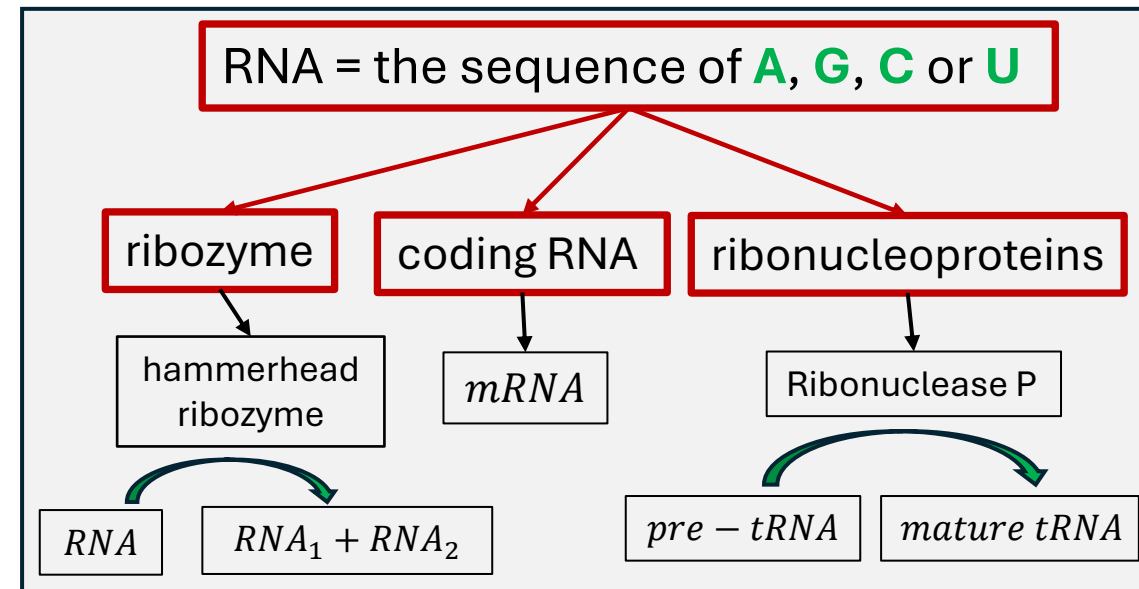
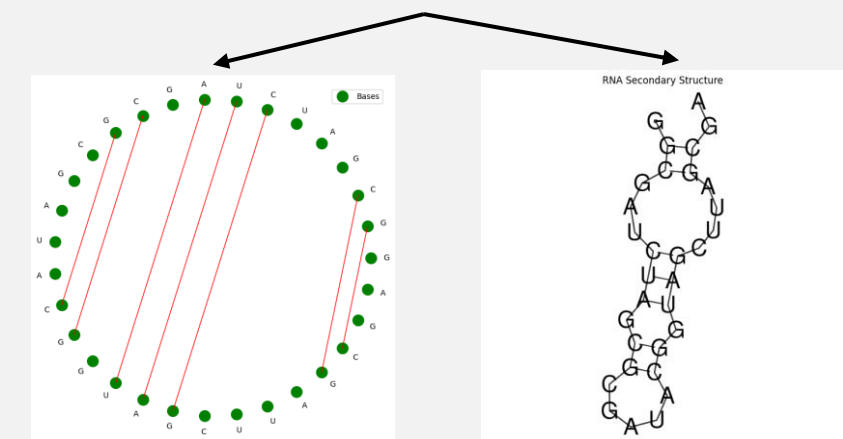
❖ 1. What's next

❖ 2. What is possible destination: Hairpins or ssRNA ?

What is RNA molecule ?



	Adenine	Guanine	Cytosine	Uracil
O	0	1	1	2
N	5	5	3	2
C	5	5	4	4
H	5	5	5	4
$\Delta_f H_{solid}^0, kJ/mol$	96.9	-183.9	-221	-424.4
$\Delta_c H_{solid}^0, kJ/mol$	-2779.0	-2498.2	-2067	-1721.3
$M_w, g/mol$	135	151	111	112
Hydrogen bonds	2	3	3	2

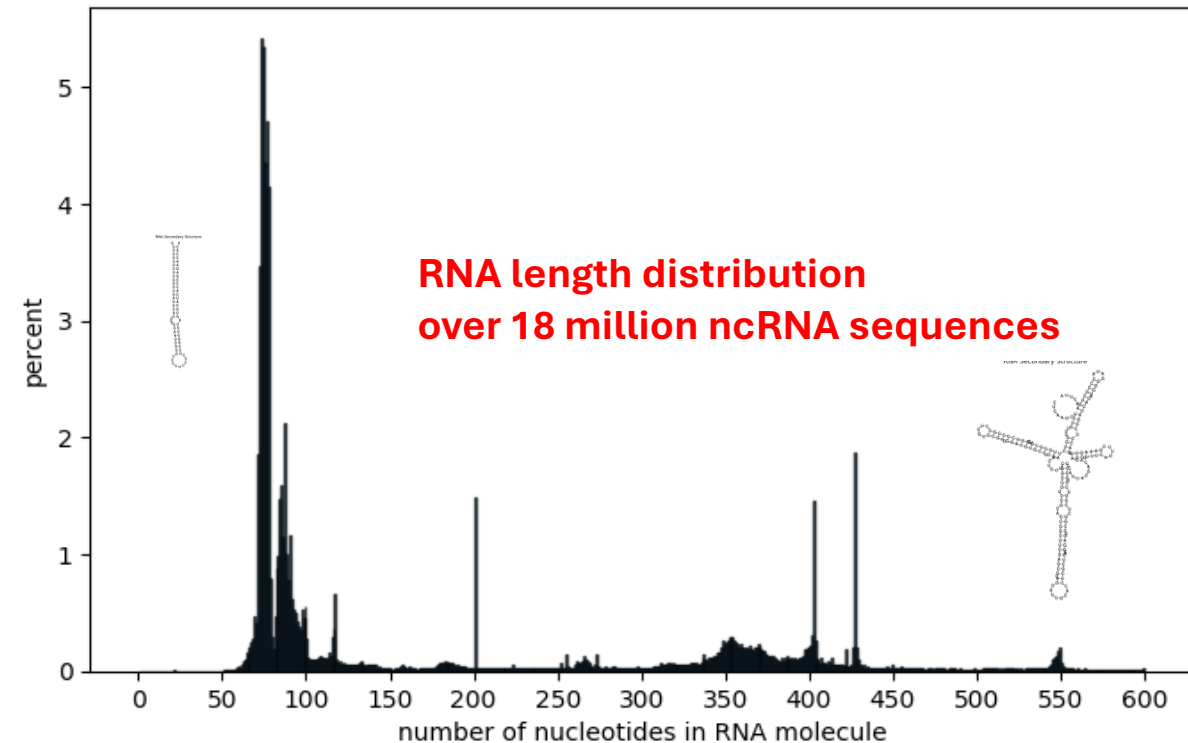
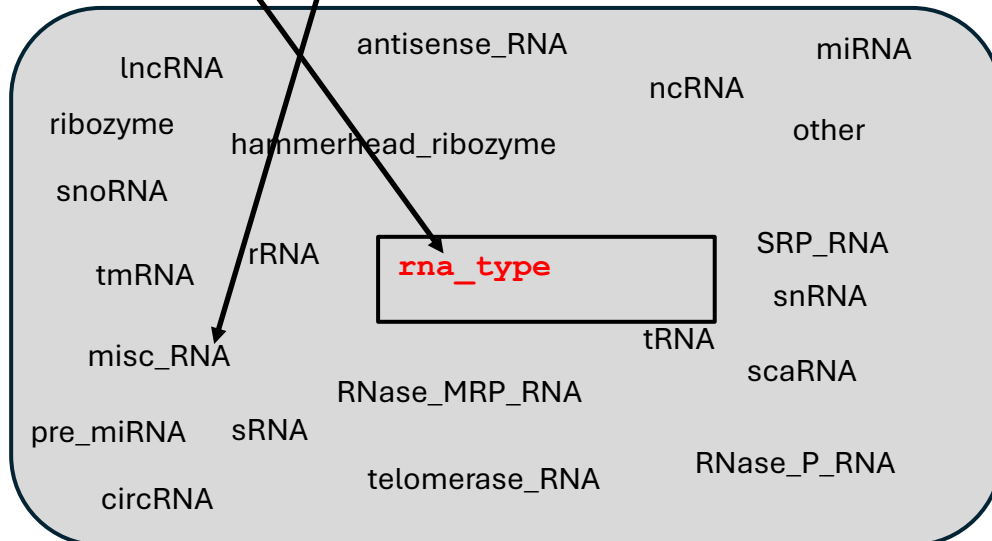
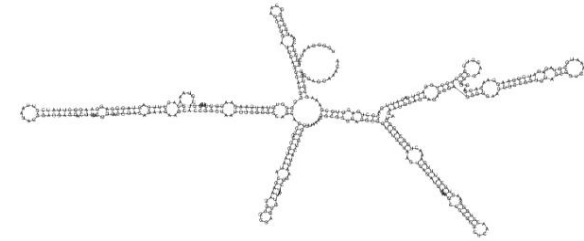


The dataset: RNACentral database

RNACentral Browsable API

https://rnacentral.org/api/v1/rna/?page=3&page_size=100 gives:

```
{ "url": "http://rnacentral.org/api/v1/rna/URS0002915621",
  "rnacentral_id": "URS0002915621",
  "md5": "fee3fe68dbd91ee898bffd9d4b89b2e9",
  "sequence": "AUGGAUGGUUGAUCAGAGAACGUACAUUUUAUAAAUGGUGUAUGUCAAUUGAUCCACAGUCCCU",
  "length": 64,
  "xrefs": "http://rnacentral.org/api/v1/rna/URS0002915621/xrefs",
  "publications": "http://rnacentral.org/api/v1/rna/URS0002915621/publications",
  "is_active": true,
  "description": "pre_miRNA from 0 species",
  "rna_type": "pre_miRNA",
  "count_distinct_organisms": 4,
  "distinct_databases": [ "Rfam" ] }, ...
```



RNA Prompt analysis using Large Language Model (**RNA alignment Tool** for RNA sequence comparison)

The answer is fully correct.

You are an expert in molecular biology. Your task is to analyze two RNA sequences and determine which is more likely to be found in a real biological organism. Justify your answer in one sentence.

Sequence 1: GGGGGGGG

Sequence 2: AAGUCGCGCCGAAAAGGUGUCUCUU

Question: Which sequence is more biologically realistic?

Answer format: One sentence explaining which sequence is more likely and why.

1. Sequence 2 is more biologically realistic because it has a more diverse nucleotide composition and is not a repetition of a single nucleotide.

Including additional context would help make the answer clearer and more specific

```
pip install biopython
from Bio.Align import PairwiseAligner
```

```
<class 'Bio.Align.Alignment'>
target      0 GAG--GCG-----GGUG----- 10
            0 .||--|||-----|||----- 25
query       0 AAGUCGCGCCGAAAAGGUGUCUCUU 25
```

The LLM has difficulty understanding this.

```
{
  "target_sequence": "GAGGCGGGUG",
  "query_sequence": "AAGUCGCGCCGAAAAGGUGUCUCUU",
  "aligned_target": "GAG--GCG-----GGUG-----",
  "aligned_query": "AAGUCGCGCCGAAAAGGUGUCUCUU",
  "score": 0.2,
  "identical_nucleotide_counts": {
    "A-A": 1,
    "G-G": 6,
    "C-C": 1,
    "U-U": 1
  }
}
```

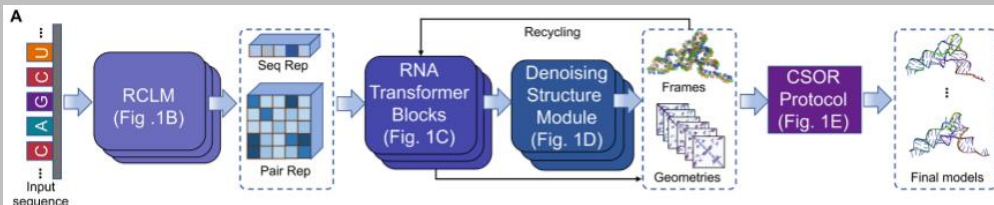
dictionaries **are better understood** by LLMs

USE for
LLM
prompt

RNA 3D structure parameters Tool:

combine DRfold2 and DSSR to extract 3D structural parameters of RNA

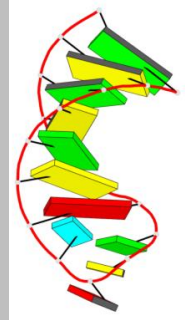
DRfold2 enables the prediction of RNA 3D structures (in PDB format) from nucleotide sequences provided in FASTA format



DRfold2 pipeline for end-to-end RNA structure prediction.

GCGCGCAUACGUGCGCGC

DRfold2



Li, Yang Li et al., Ab initio RNA structure prediction with composite language model and denoised end-to-end learning, Cold Spring Harbor Laboratory, (2025), doi = {10.1101/2025.03.05.641632}

DSSR

API: <http://skmatic.x3dna.org/api>

```
{
  "general_info": {
    "full_RNA_sequence": "GCGCGCAUACGUGCGCGC",
    "length": 18,
    "base_pairs": 7,
    "hydrogen_bonds": 25,
    "dot_bracket": [
      "(((((((.....)))))))))"
    ],
    "splayUnits": [
      "UA"
    ],
    "hairpins": [
      "AUACGU"
    ],
    "stacks": [
      "AU",
      "ACGU"
    ]
  },
  "helices_info": {
    "full_RNA_sequence": "GCGCGCAUACGUGCGCGC",
    "helix_0": {
      "base_pairs": 7,
      "strand_1": "GCGCGCA",
      "strand_2": "CGCGCGU",
      "helix_form": "AAAAAA"
    }
  }
}
```

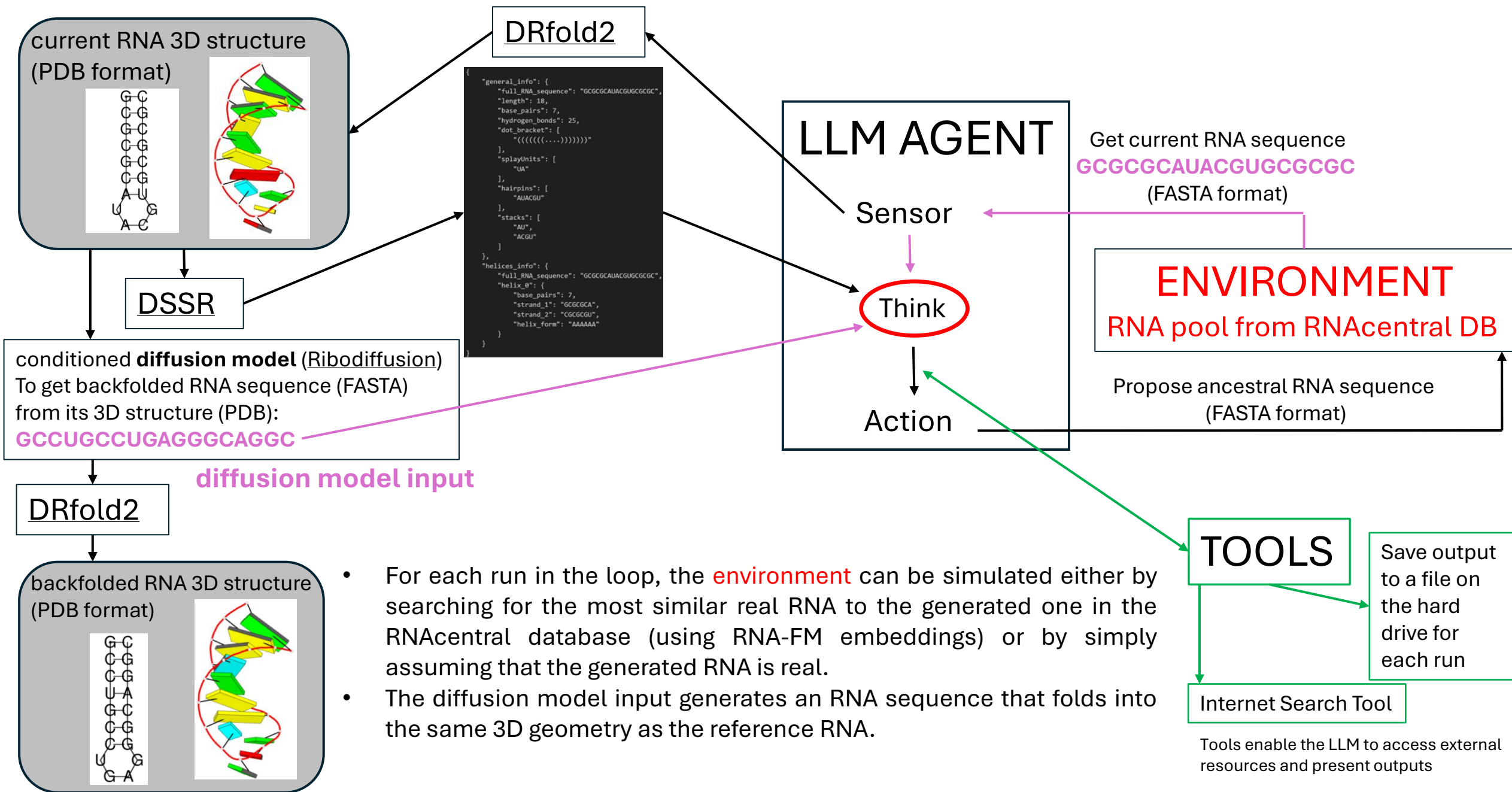
Xiang-Jun Lu, DSSR-enabled innovative schematics of 3D nucleic acid structures with PyMOL, Nucleic Acids Research, Vol 48, number 13, p. e74-e74, (2020), doi = {10.1093/nar/gkaa426}

Input

Output for LLM input

From RNA sequence to a parametric description of its 3D structure

LLM Tool-Agent Computational Pipeline



- For each run in the loop, the **environment** can be simulated either by searching for the most similar real RNA to the generated one in the RNAcentral database (using RNA-FM embeddings) or by simply assuming that the generated RNA is real.
- The diffusion model input generates an RNA sequence that folds into the same 3D geometry as the reference RNA.

Prompt Engineering: Designing Effective Inputs for LLMs

```
prompt = ChatPromptTemplate.from_messages([
    (
        "system",
        """
        You are a research assistant that will help generate a research paper.
        Answer the user query and use necessary tools.
        Wrap the output in this format and provide no other text:
        {format_instructions}
        Do not include any other commentary or explanation — output only the JSON.
        """,
    ),
    ("placeholder", "{chat_history}"),
    (
        "human",
        """{query}""",
    ),
])
```

Current RNA sequence:
{current_sequence}

3D Structural Parameters of the current RNA:
{rna_structure_block}

Backfolded RNA sequence from 3D structure of the current RNA:
{backfolded_sequence}

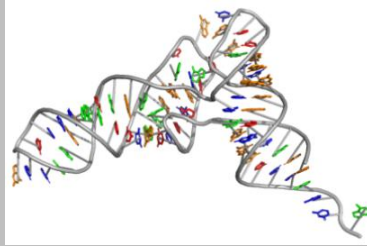
```
""",
    ),
    ("placeholder", "{agent_scratchpad}"),
]
).partial(format_instructions=parser.get_format_instructions())
```

```
query = (
    f"Propose a plausible ancestral RNA sequence based on the current RNA sequence and its properties."
    f"Save both a summary and the proposed ancestral_sequence to a file"
    f"and begin each output in the file with the following section header: evolution_step_{i}"
    raw_response = agent_executor.invoke({"query": query,
        "current_sequence": current_sequence,
        "backfolded_sequence": backfolded_sequence,
        "rna_structure_block": rna_structure_block})
)
```

No conditions were imposed on the generated ancestral RNA sequence (e.g., limits on length or number of mutation events per step)!

STEP 1

GGACCGAUAGUCUCGUGGUAGGAACUUAACCCUGCCAAGG
AUAGAGCCCGGGUUCGAAUCCGGGUCGGUCCACCA



DSSR output

```
length: 75
base_pairs: 29
hydrogen_bonds: 112
dot_bracket: ((((((((((.....)))).(((.....)))).((((.....))))))))......
multiplets: UUG, CGG
splayUnits: AU, UG, GUA, CC, AA, CA
ssSegments: ACCA
junctions: AUAGCUUAGAGCGU
hairpins: CGUGGUAG, CCUGCCAAG, GUUGCAUIC
nonStack: UGUUGA
stacks: AC, GG, CU, GC, CG, AC, GU, UCU, UGG, GAA, AAC, CCU, GAU, GGG, GUU, GACC, AAGA, ACAU, CAG
num_coaxStacks: 2
helix_0: base_pairs=12, strand_1=GGACCGACCGGG, strand_2=CCUGCGUGGCCG, helix_form=AAAAAXAA.A
helix_1: base_pairs=13, strand_1=CAGGAGUAGUCUG, strand_2=UCCCAAUCAAGGA, helix_form=X.A.XAA.x
```

The provided 75-nucleotide RNA sequence exhibits characteristic tRNA-like structural features with a cloverleaf secondary structure. Key structural parameters include:

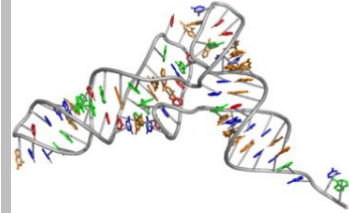
- Length: 75 nucleotides
- Base pairs: 29
- Dot-bracket notation showing complex stem-loop structures
- Multiple hairpins and junction regions
- Two coaxial stacks indicating sophisticated tertiary interactions

The first two images in each panel were generated using simplified 2D structures for rough visualization purposes only.

The last two images depict the actual 3D structures, predicted by DRfold2 and further processed by DSSR to extract structural parameters for LLM input.

ANCESTRAL_SEQUENCE:

GGCCCGAUAGCUCGUGGUAGGAACUUAACCCUGCCAAGGAUAGAG
CCCGGGUUCGAAUCCGGGUCGGGCCACCA



Rationale for Ancestral Sequence Reconstruction

1. ****Structural Conservation****: The ancestral sequence maintains the essential cloverleaf structure characteristic of early tRNA-like molecules, preserving critical base-pairing regions that ensure functional stability.
2. ****Reduced Complexity****: Early RNA molecules likely had simpler tertiary structures with fewer modified nucleotides and less sophisticated coaxial stacking arrangements.
3. ****Enhanced Thermostability****: Ancestral RNA sequences typically exhibited greater thermodynamic stability to survive in harsh primordial conditions, achieved through stronger base-pairing interactions.
4. ****Functional Core Preservation****: The proposed ancestral sequence maintains the essential functional domains including the acceptor stem, anticodon loop region, and critical junction sequences necessary for aminoacylation and ribosome binding.
5. ****Evolutionary Parsimony****: The reconstruction follows the principle of minimal evolutionary changes while preserving essential structural and functional characteristics required for early protein synthesis machinery.

The proposed ancestral sequence represents a plausible evolutionary precursor that could have given rise to the current RNA through gradual structural refinements and optimization for specific cellular environments.

Based on analysis of the current RNA sequence (75 nucleotides) with its complex secondary structure containing 29 base pairs, multiple hairpins, and helical regions, I propose an ancestral sequence that would have been simpler yet structurally viable. The current sequence shows sophisticated features including two major helices, multiple hairpin loops, and complex junction regions.

For the ancestral reconstruction, I considered several evolutionary principles:

1. Ancient RNA sequences likely had simpler secondary structures with fewer complex loops
2. Early RNA would have had stronger base-pairing stability in core regions
3. Primitive sequences would have contained more canonical Watson-Crick pairs
4. The ancestral form would retain essential structural motifs but with reduced complexity

The proposed ancestral sequence maintains the basic cloverleaf-like structure but with:

- Simplified hairpin loops with more stable base-pairing
- Reduced junction complexity
- Stronger stem regions with canonical base pairs
- Shorter variable loops
- Enhanced stability through increased GC content in critical structural regions

This ancestral form would represent an evolutionary intermediate that possessed the fundamental structural framework while being simpler and more thermodynamically stable than the modern sequence.

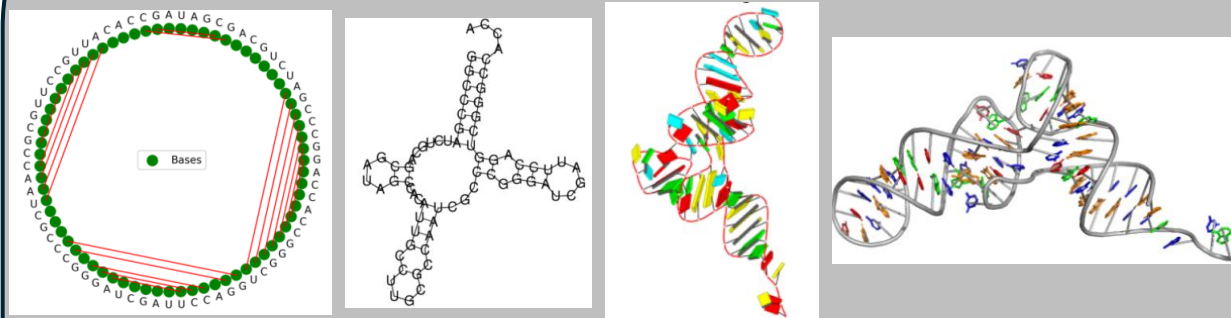
Current-Ancestral RNA sequences alignment

```
<class 'Bio.Align.Alignment'>  
target      0 GGACCAGUAGUCUCUGGUAGGAAACUAACCCUGCCAAGGAIAGAAGCCGGGUUCGAU  
            0 |||||-----|||||  
query       0 GGGCCAGUAG -CUCUGGUAGGAAACUAACCCUGCCAAGGAIAGAAGCCGGGUUCGAU  
  
target      60 CGGGUCGGUCCACCA 75  
            60 |||||.|.|||. 75  
query       59 CGGGUCGGGCCACCA 74  
  
Score: 140.0
```

To improve the reliability of these evolutionary trajectories, it is necessary to introduce constraints on the number of mutation events per step.

STEP 6

GGCCCGAUCUGCAGCGAUAGCCACAUUGCCUUGCGCCAAUCGCC
CGGGAUCGAUUCCAGGUCGGGCCACCA



DSSR output

```
length: 71
base_pairs: 32
hydrogen_bonds: 111
dot_bracket: (((((((.....((.....))....(((.....))....(((.....))....(((.....))....))))))....
multiplets: CGC, UGCG
splayUnits: UC, GAUA, UU, CC, UU, CC
ssSegments: ACCA
junctions: AUCUGCGCCACAUCGCGU
hairpins: CGAUAG, CUUGCG, AUCGAUU
nonStack: AUUU
stacks: CU, UC, CG, AU, CA, GU, CA, GAC, AUC, GGC, CAG, CGU, GGG, CCAC, GCCU, UGCGCC
num_coaxStacks: 1
helix_0: base_pairs=13, strand_1=GGCCCGACCGGA, strand_2=CGGGGUGGACCU, helix_form=AAAAAxA, .AA
helix_1: base_pairs=8, strand_1=UGAUGCU, strand_2=GUACGC, helix_form=.xAAAx.
helix_2: base_pairs=5, strand_1=CAGCG, strand_2=ACGCG, helix_form=.Ax
```

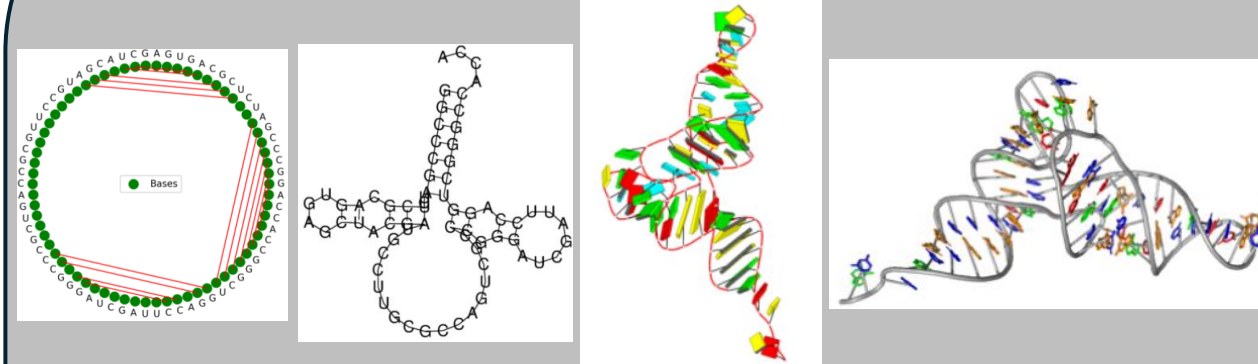
Based on the analysis of the current RNA sequence (71 nucleotides) and its structural properties, I propose an ancestral RNA sequence that maintains the critical structural elements while reflecting probable evolutionary simplifications. The current RNA displays a complex secondary structure with 32 base pairs, 3 major helices, and multiple structural motifs including hairpins, bulges, and junctions.

Key observations from the current sequence:

- Length: 71 nucleotides
- Three major helices (13, 8, and 5 base pairs respectively)
- Complex dot-bracket notation indicating nested stem-loop structures
- Multiple hairpin loops (CGAUAG, CUUGCG, AUCGAUU)
- Junction regions and single-stranded segments
- Strong GC content in stem regions providing stability

ANCESTRAL_SEQUENCE:

GGCCCGAUCUCGCAGUGAGCUACGAUGCCUUGCGCCAGUCGCCC
GGGAUCGAUUCCAGGUCGGGCCACCA



The proposed ancestral sequence likely represents a simpler evolutionary precursor that:

1. Maintains the core three-helix architecture
2. Has simplified loop regions with fewer complex motifs
3. Retains essential base-pairing patterns for structural stability
4. Shows reduced sequence complexity while preserving functional domains
5. Exhibits characteristics consistent with early RNA world molecules

The ancestral reconstruction considers:

- Conservation of critical structural elements
- Simplification of complex motifs that likely evolved later
- Maintenance of thermodynamic stability
- Preservation of potential catalytic or binding sites
- Evolutionary parsimony in sequence changes

```
<class 'Bio.Align.Alignment'>
target      0 GGCCCGAUCU-GCAGCGAUAGCCACAUUGCCUUGCGCCAAUCGCCCGGGAUCGAUCCAG
              0 |||||---|||..--|||..||..|||||..|||||
query       0 GGCCCGAUCUCGAGUG--AGCUACGAUGCCUUGCGCCAGUCGCCGGGAUCGAUCCAG

target      59 GUCGGGCCACCA 71
              60 ||||| 72
query       58 GUCGGGCCACCA 70

Score: 118.5
```

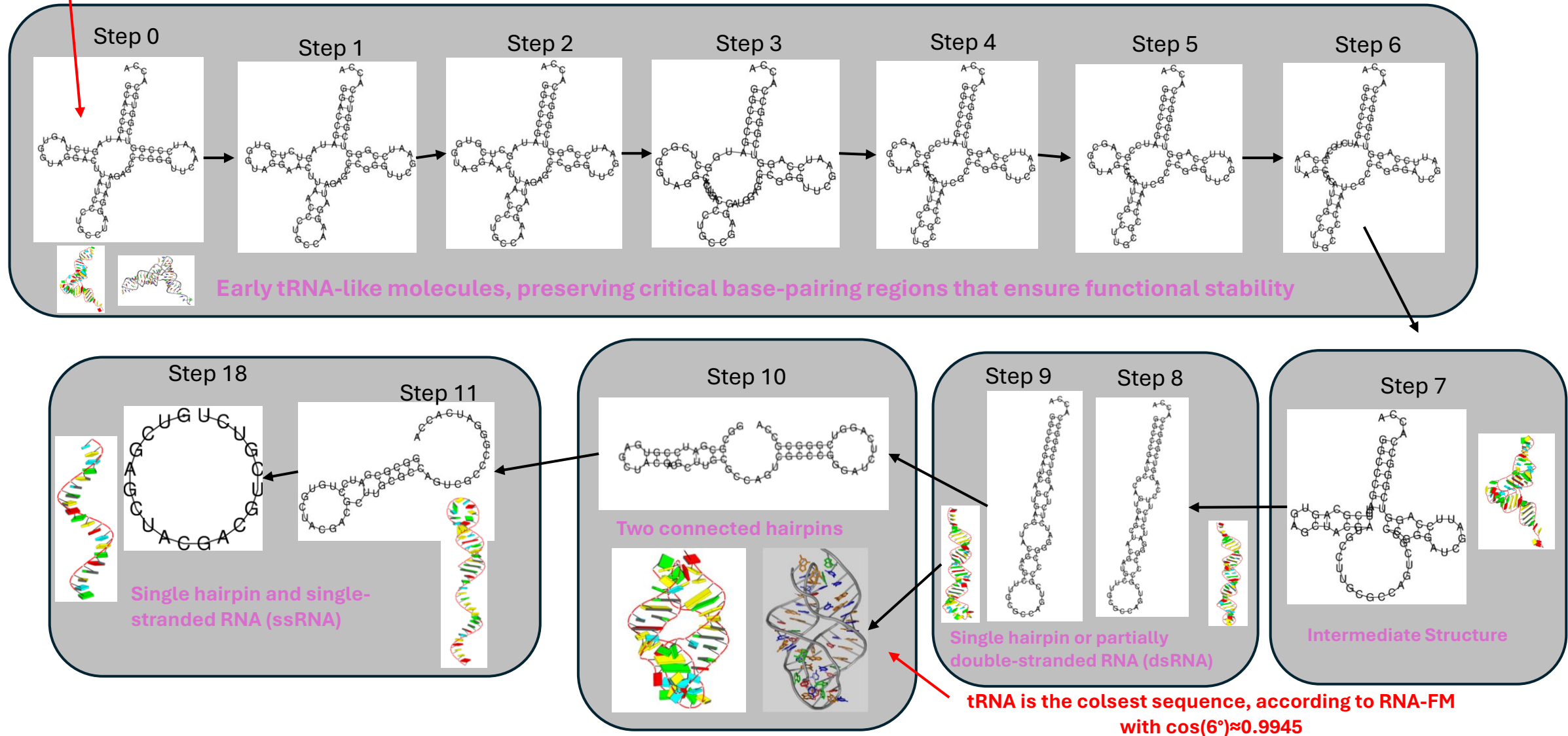
The first two images in each panel were generated using ViennaRNA package for rough visualization purposes only.

The last two images depict the actual 3D structures, predicted by DRfold2 and further processed by DSSR to extract structural parameters for LLM input

To improve the reliability of these evolutionary trajectories, it is necessary to introduce constraints on the number of mutation events per step.

Results of Simulated Reverse Evolution of tRNA^{Gly} Asgard_group_archaeon (First Run)

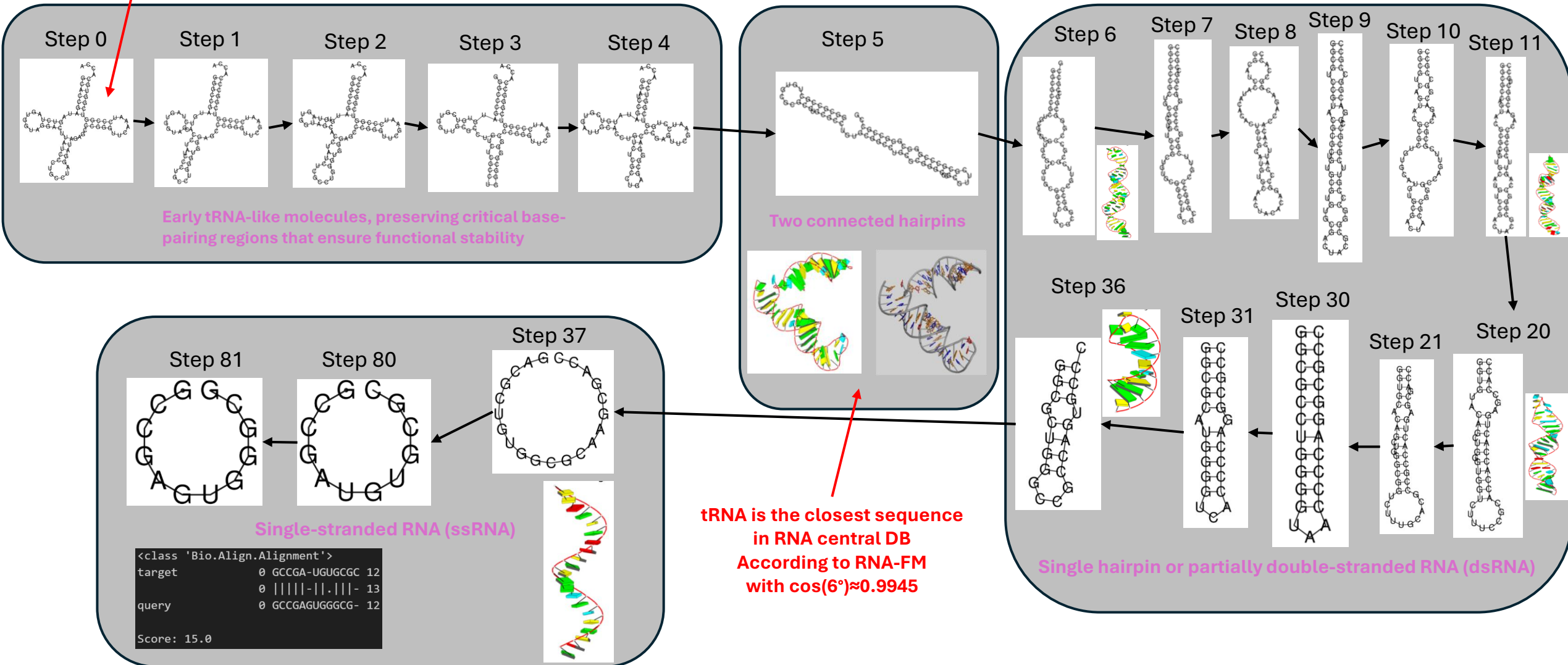
tRNA^{Gly}
Asgard_group_archaeon
Starting point



To improve the reliability of these evolutionary trajectories, it is necessary to introduce constraints on the number of mutation events per step.

Results of Simulated Reverse Evolution of tRNA^{Gly} Asgard_group_archaeon (Second Run)

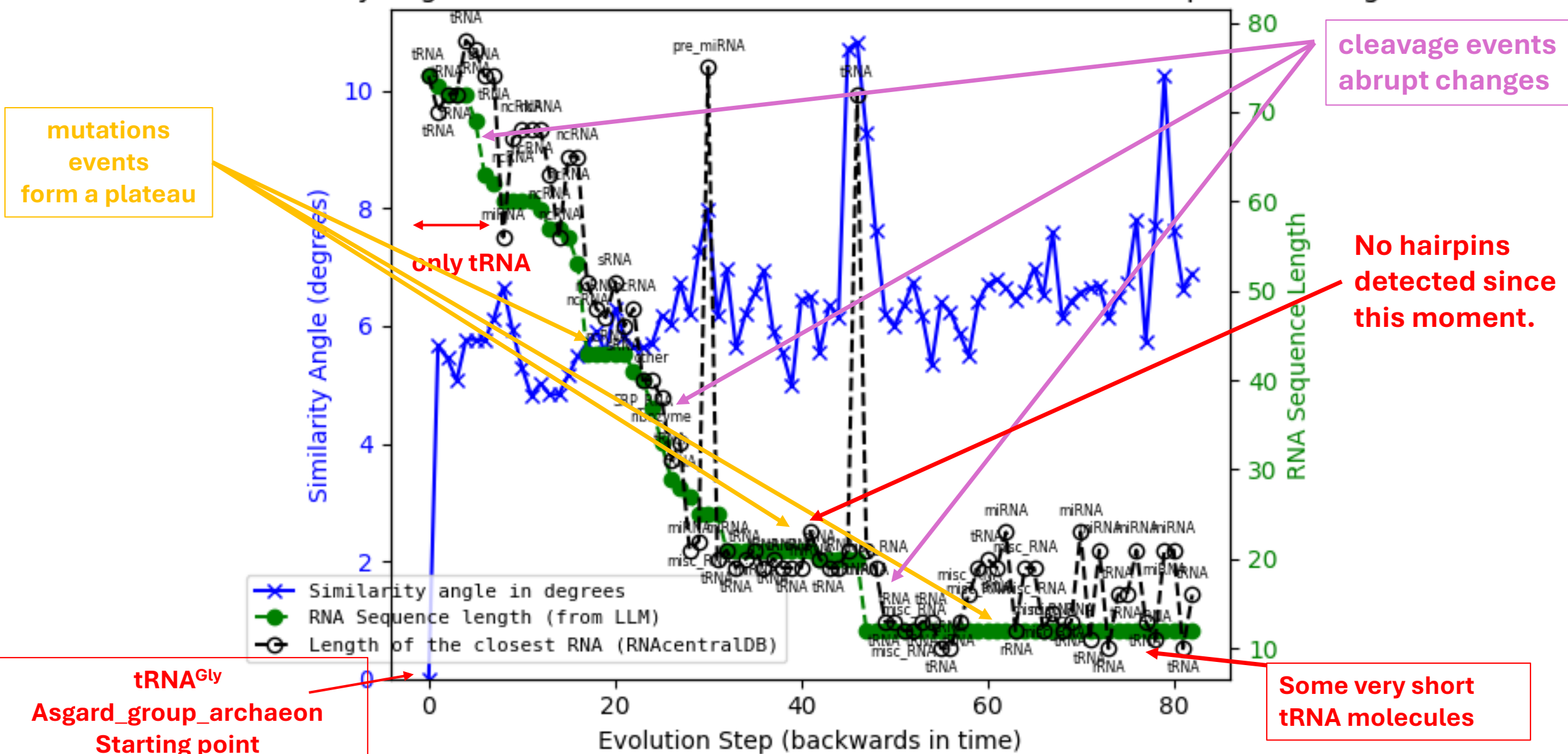
tRNA^{Gly}
Asgard_group_archaeon
Starting point



To improve the reliability of these evolutionary trajectories, it is necessary to introduce constraints on the number of mutation events per step.

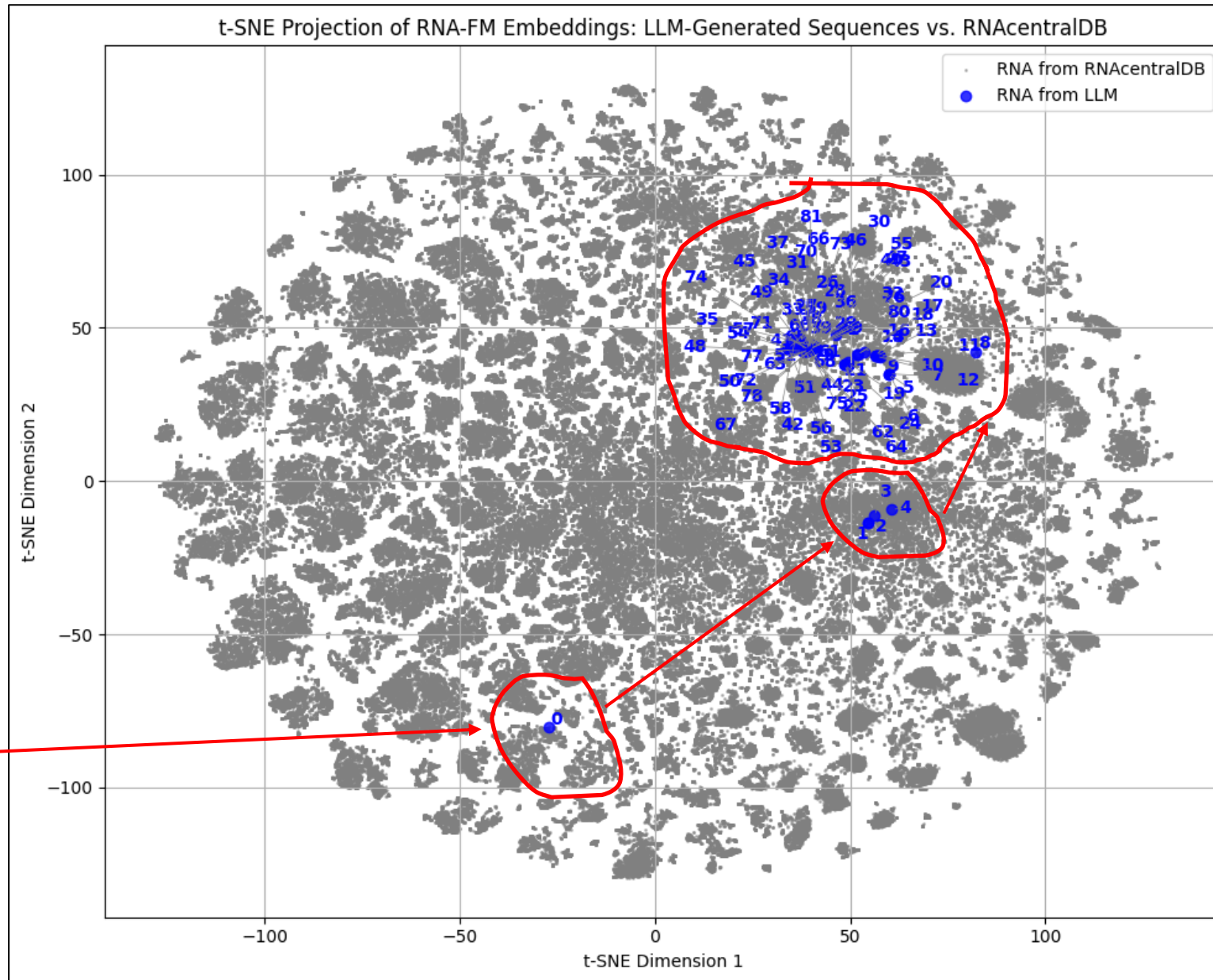
Using Large Language Models to Simulate the Reverse Evolution of tRNA^{Gly}

Smallest similarity angle between RNAs from LLM and RNACentralDB (comparison using RNA-FM)



From tRNA^{Gly} to short tRNA and miscellaneous RNA

How Accurately do LLM-Designed RNAs Represent Real RNA?

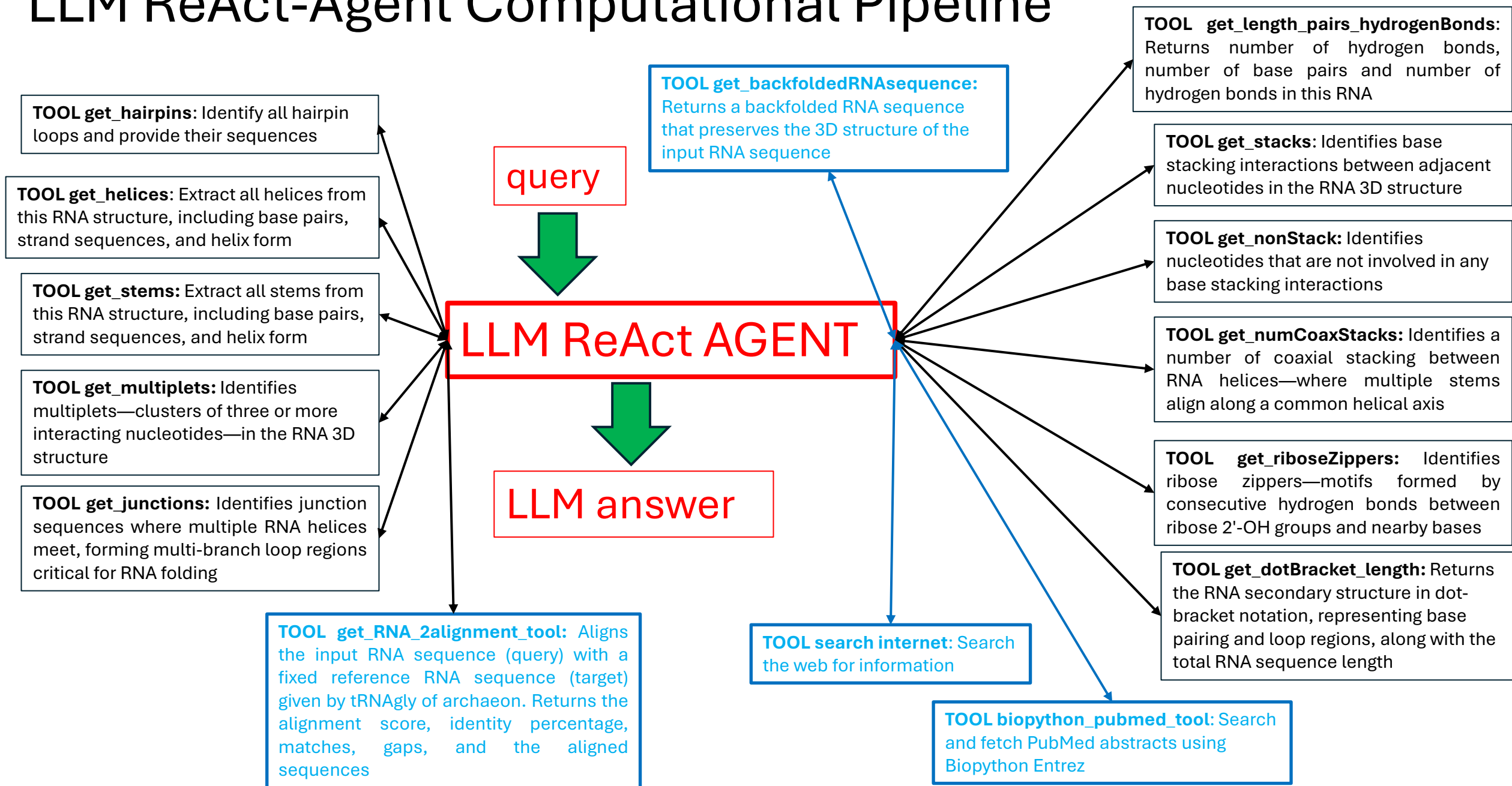


0, 1, ... are
evoution step
(backwards in
time)

tRNA^{Gly}
Asgard_group_archaeon
Starting point

400,000 tRNA sequences from the RNAcentral database with lengths less than 80 nucleotides

LLM ReAct-Agent Computational Pipeline



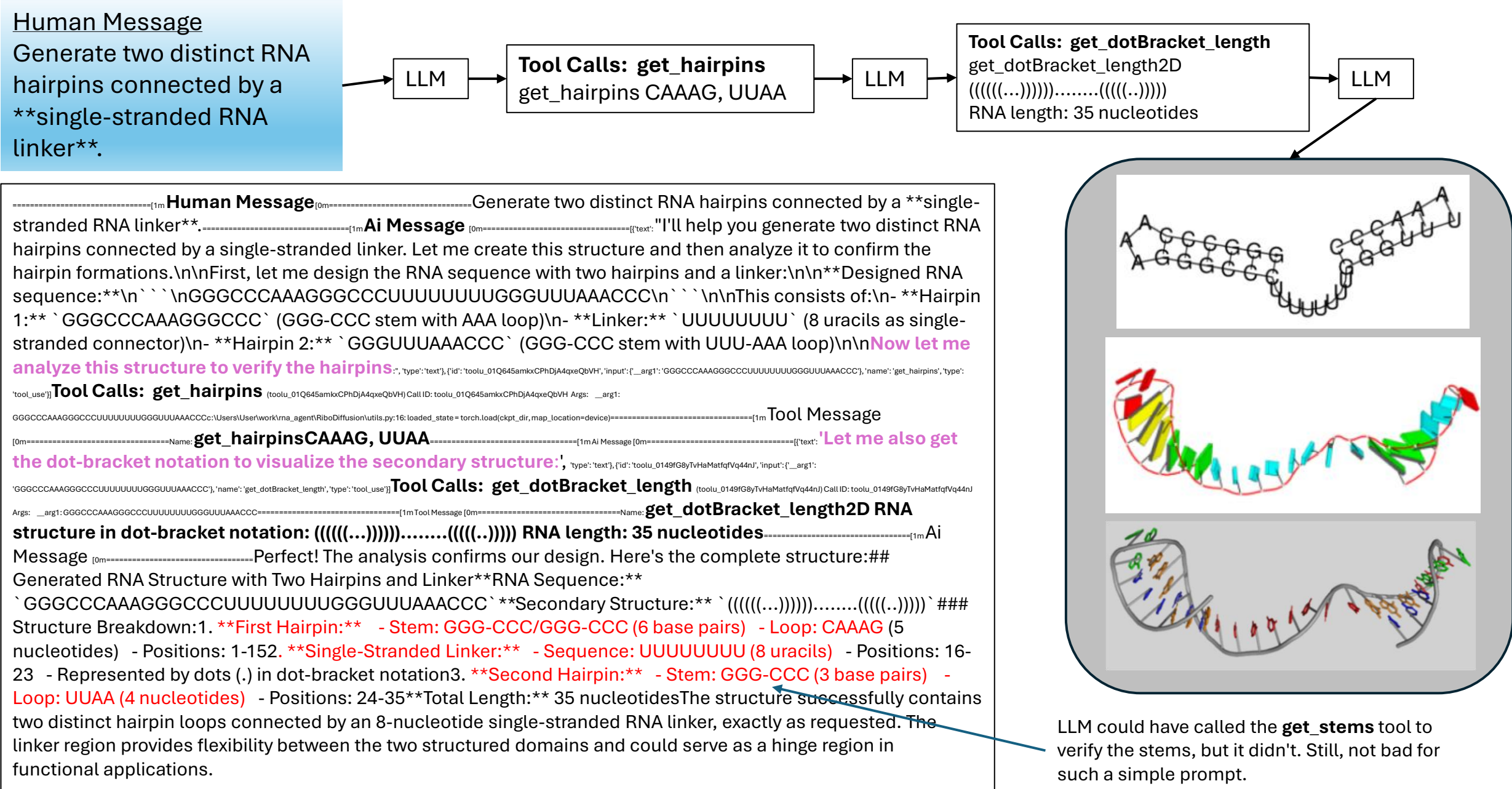
Agent: Comparison of Two RNA Sequences



Provide the output in clearly labeled sections.

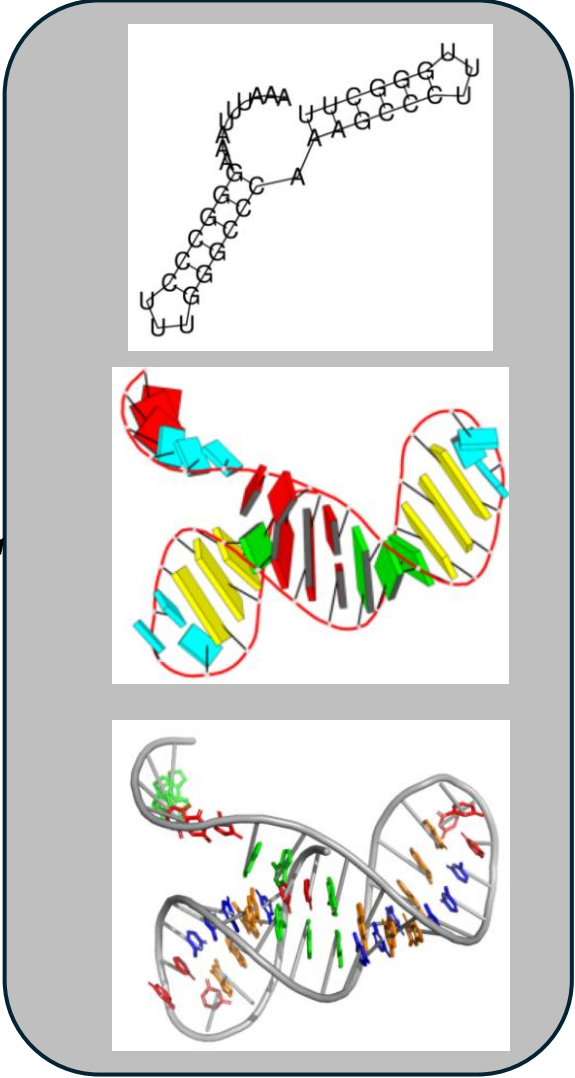
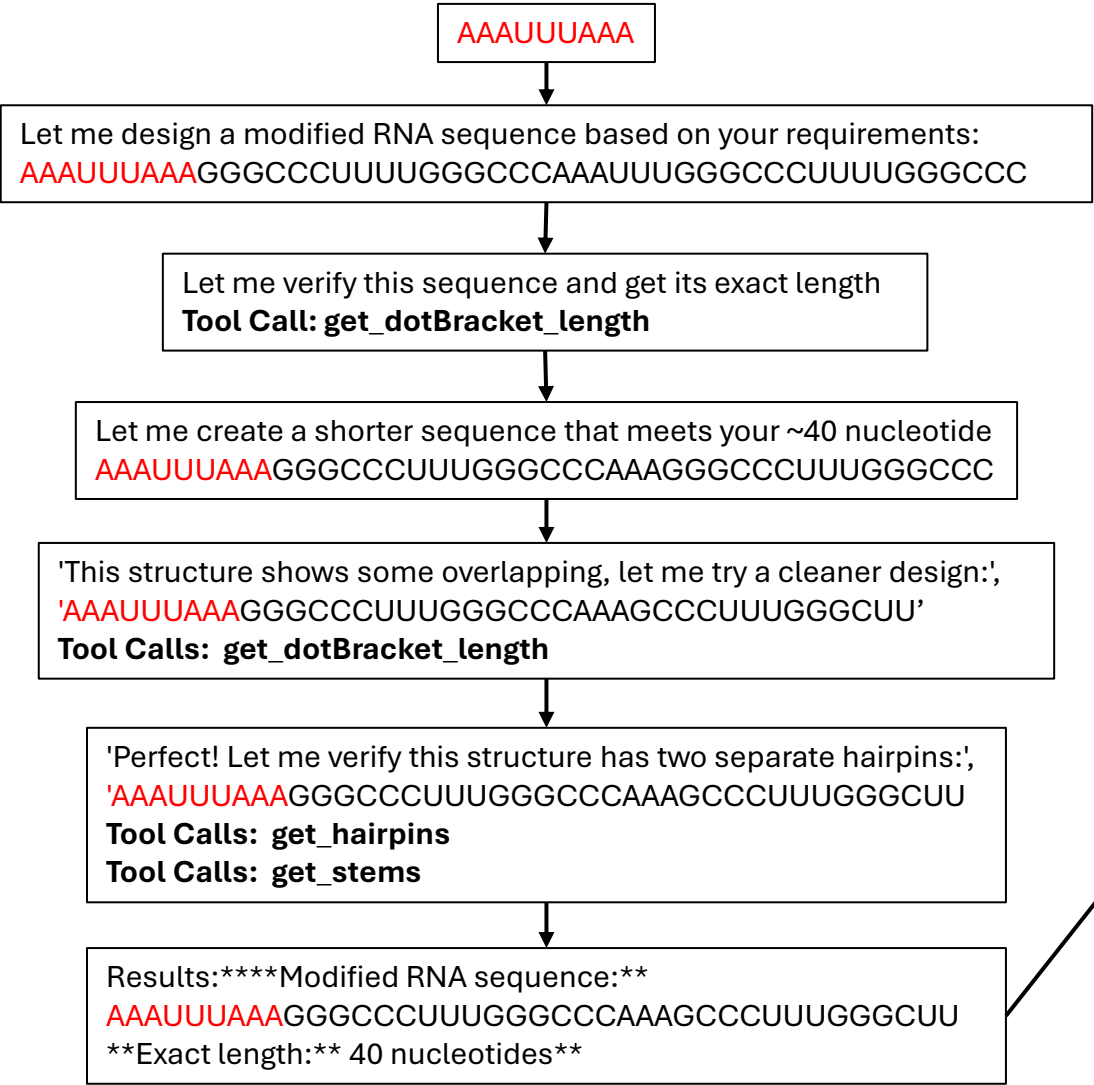
Aspect Query (AG repeat) Target (tRNA-like)
----- ----- -----
Length 20 nt 74 nt
Structure Completely unstructured Highly structured (33 base pairs)
Complexity Simple alternating repeat Complex with 4 helices, 3 hairpins
Function Synthetic/artificial Functional tRNA-like molecule
Stability Low (no base pairs) High (123 hydrogen bonds)
Evolution Likely artificial Biologically evolved structure
Diversity

LLM ReAct agent : Generate Two Connected RNA Hairpins without a Prior RNA Template



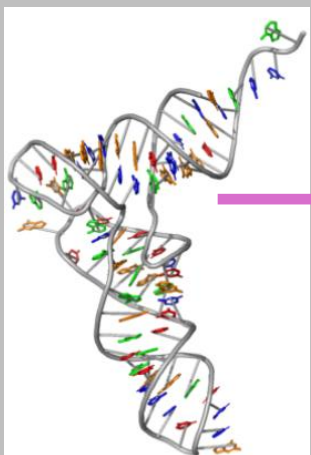
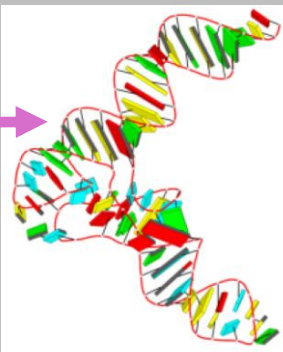
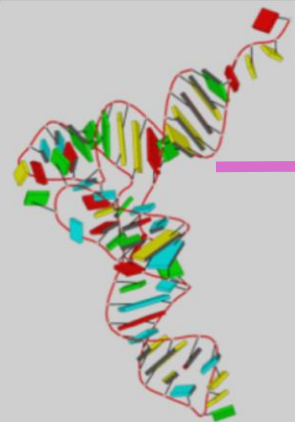
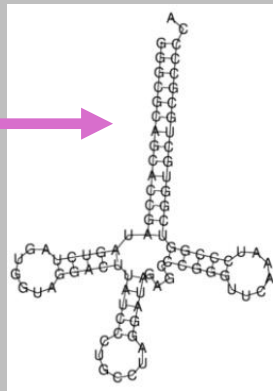
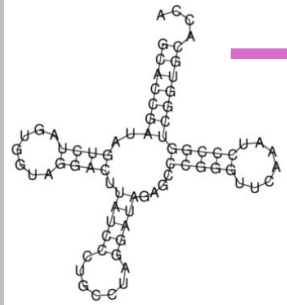
LLM ReAct agent : Generate Two Connected RNA Hairpins with a Prior RNA Template

The reference sequence (input): **AAAUUUAAA**
Modify this reference sequence to form a structure without ****pseudoknot base pairs**** consisting of ****two separate hairpins**** connected by a ****single-stranded RNA linker****.
The modified RNA sequence must be about 40 nucleotides long.Return the modified RNA sequence and its exact length.



reference sequence modified sequence

tRNA^{Gly}
Asgard_group_archaeon



LLM ReAct Agent: Modification of a Specified Region of an RNA Molecule

f"Current tRNA sequence (input): {tRNA}\n\n"

"Modify this reference sequence by increasing the length of the acceptor stem to ****twice its original size****. "

"Ensure that ****no more than 20%**** of the nucleotides are changed or added.\n\n"

"Keep the rest of the tRNA unchanged.\n\n"

"Verify the structure and return the modified RNA sequence."

Current tRNA sequence (input):

GCACCGAUAGUCUAGUGGUAGGACUUAUCCUGCCUAGGAUAGAGCCCGGGUCAAUCCCGGUCGGUGCACCA

I'll help you modify the tRNA sequence by doubling the acceptor stem length while keeping changes to no more than 20% of nucleotides. First, let me analyze the current structure to identify the acceptor

Tool Calls: **get_dotBracket_length**

'Now let me identify the stems to locate the acceptor stem

Tool Calls: **get_stems**

'Let me also get the hairpin loops to better understand the structure

Tool Calls: **get_hairpins**

Based on the analysis, I can see this is a standard tRNA structure with:\n- ****Acceptor stem****: **helix_0 with 7 base pairs (GCACCGA paired with CGUGGCU)**\n- **D-arm**: **helix_1 with 4 base pairs**\n- **Anticodon arm**: **helix_2 with 5 base pairs** \n- **TψC arm**: **helix_3 with 5 base pairs**\n\nTo double the acceptor stem length from 7 to 14 base pairs while changing ≤20% of nucleotides (≤15 out of 74), I'll:\n1. Extend the 5' end with 7 complementary base pairs\n2. Extend the 3' end with 7 complementary base pairs\n3. Keep all other structural elements unchanged\n\nHere's the modified sequence: **GGGC...**

Tool Calls: **get_dotBracket_length**

Tool Calls: **get_stems**

Figure adapted from Frontiers in Molecular Biosciences (Irem Avclar-Kucukgoze et al., 2020), CC BY 4.0. DOI: 10.3389/fmolb.2020.610617

Perfect! The modification is successful:## Summary:- ****Original length****: 74 nucleotides- ****Modified length****: 86 nucleotides (12 nucleotides added = 16.2% increase, well under 20% limit)- ****Acceptor stem****: **Successfully doubled from 7 to 14 base pairs**- ****Structure integrity****: All other arms (D-arm, anticodon arm, TψC arm) remain unchanged- ****Base pair complementarity****: Maintained throughout the extended acceptor stem## Modified tRNA sequence:**`GGGCGCAGCACCGAUAGUCUAGUGGUAGGACUUAUCCUGCCUAGGAUAGAGCCCGGGUCAAUCCCGGUCGGUGCUGCGCCCCA`**The acceptor stem is now twice its original length (14 bp vs 7 bp) while preserving the overall tRNA secondary structure and staying within the 20% nucleotide change constraint.

CONCLUSIONS

- ❖ **An LLM agent can be used not only to generate new RNA sequences but also to modify existing ones, offering an advantage over conditional diffusion models alone when guiding RNA design.**
- ❖ **A preliminary attempt to model RNA reverse evolution shows a decrease in RNA length and identifies three distinct evolutionary patterns:**
 - The ancestral sequence maintains the essential cloverleaf structure characteristic of early tRNA-like molecules, preserving critical base-pairing regions.
 - A cloverleaf structure transforms into a single hairpin or partially double-stranded RNA (dsRNA) through intermediate structures (e.g., two connected hairpins).
 - Finally, the hairpin or dsRNA transforms into single-stranded RNA (ssRNA).
- ❖ **To improve the reliability of these evolutionary trajectories, it is necessary to introduce **constraints** on the number of mutation events per step. Additional ideas are presented on the next three slides.**

1. What's next ?

1. Introduce **limits** on the length or **the number of mutation events per step**
2. Search for mistakes in the answers and correct the prompt accordingly

3. Get more context from answering questions about RNA sequences

They effectively used the RNA-FM sequence encoder to embed RNA sequences for alignment with natural language

Only RNA Sequence (e.g., "AUGGCUUAGCU...")

The Question (e.g., "What is the function of this RNA?")

RNA-GPT

answer

at this time, **RNA-GPT** is not freely available for use

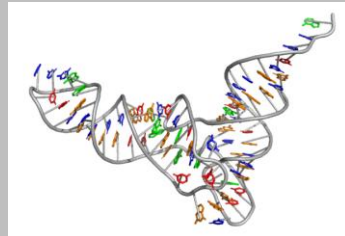
Yijia Xiao et al. , RNA-GPT: Multimodal Generative System for RNA Sequence Understanding, **2025**

4. Get more context from answering questions about RNA images?

HealthGPT is a medical large vision-language model for unifying comprehension and generation via heterogeneous knowledge adaptation

HealthGPT **understands medical images** (e.g. answer questions about them)

Q: What type of RNA molecule is shown in the image?



HealthGPT

This is tRNA not rRNA...

HealthGPT-M3 is available

HealthGPT-L14 weights are still not released yet

ANSWER of HealthGPT-M3 : The image shows a **ribosomal RNA (rRNA)** molecule. Ribosomal RNA is a type of RNA that is a key component of the ribosome, the cellular machinery responsible for protein synthesis. The image depicts the secondary structure of the rRNA molecule, which is formed by the folding and pairing of the RNA strands.

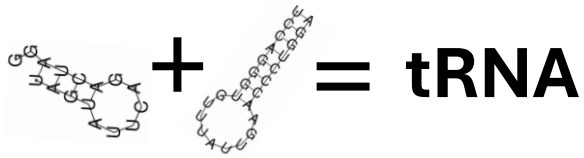
Tianwei Lin et al., HealthGPT: A Medical Large Vision-Language Model for Unifying Comprehension and Generation via Heterogeneous Knowledge Adaptation, **2025**

2. What is possible destination: Hairpins ?

Peptidyl Transferase Center (PTC) Sequences

the idea

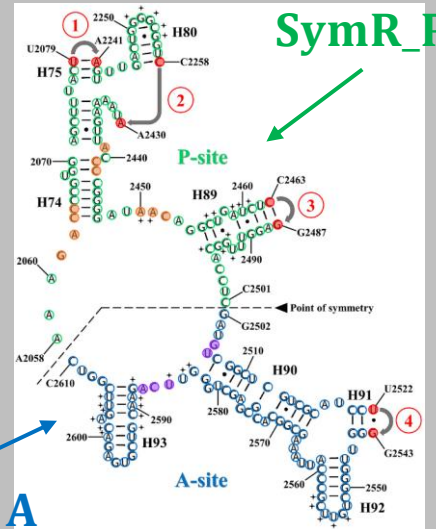
the dimerization of two similar RNA structures



“The peptidyl transferase center (PTC) evolved from a primitive system in the RNA world comprising tRNA-like molecules formed by **duplication of minihelix-like small RNA**”

Tamura, J. Biosci, 2011

pseudosymmetrical region

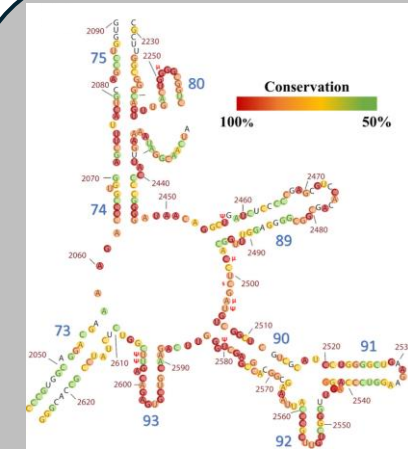


SymR_A

SymR_PA = SymR_P + SymR_A

Secondary structure of the pseudosymmetrical region (**SymR**; Agmon et al., 2005), derived from the LSU secondary structure of *Thermus thermophilus* (Petrov et al., 2013). (Madhan R. Tirumalai et al., 2021)

PTC



PTC2 = red
PTC3 = PTC2 + orange
PTC4 = PTC3 + yellow
PTC5 = PTC4 + green

Nucleotide CONSERVATION level:

Red circles: 100% conservation (78 nt).
Orange circles: 90 to 99.9% conservation (68 nt)
Yellow circles: 70 to 89.9% (52nt)
Green circles: 50 to 69.9% conservation (49nt)
Black letters: less than 50% conservation (35nt)

(Bernier et al.; Faraday Discuss, 2014)
(Madhan R. Tirumalai et al., 2021)

SymR_P is older than SymR_PA
PTC2 is older than PTC3, PTC4, PTC5

rRNA evolution: Age Variability Across Different Regions



Last Universal Common Ancestor (LUCA)

E

● Phase 6
● Phase 5
● Phase 4

LSU and SSU associated at the end of Phase 3

● Phase 3
● Phase 2
● Phase 1

LSU and SSU evolved independently & uncorrelated through Phases 1-3

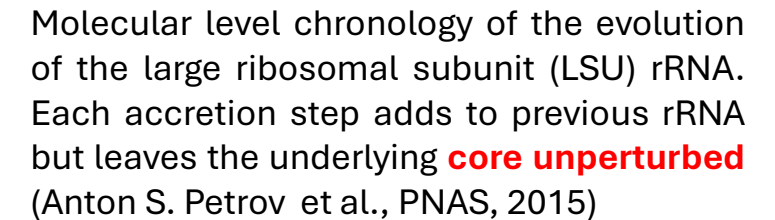
Phase 5 Phase 3 Phase 4 Phase 6

PTC Phase Phase Phase

The diagram shows a complex network of colored lines representing the LSU of tRNA in E. coli. The lines are color-coded by phase: Phase 6 (red), Phase 5 (orange), Phase 4 (yellow), Phase 3 (green), Phase 2 (light blue), and Phase 1 (dark blue). A legend on the left explains the phases and the relationship between LSU and SSU. A blue arrow points from the text 'LSU and SSU associated at the end of Phase 3' to a specific region of the diagram. A blue box highlights a region labeled 'PTC' and 'Phase'.

LSU of tRNA (E. coli):

Blue part is the oldest one



This symmetry (**SymR**) suggests that the ancient ribosome may have been **a dimer of identical or nearly identical RNA molecules**, later evolving into the asymmetrical modern ribosome with **PTC**.