# RNA Sequence Analysis Using Transformer Models

**Work in progress!**

Pavel Polyakov

# OUTLINE

- **Objectives**

- **The idea of transformers**
- **Dataset**
  - What is RNA molecule
  - RNAcental database
- **RNA autoencoder**
  - Vanilla transformer*
  - BERT-like transformer*
  - BERT-like transformer (RNA-FM model)

- **Results**
  - Word embeddings arithmetic
  - Peptidyl Transferase Center (PTC): structure and evolution

- **Conclusions**
- **What's Next?**

***https://github.com/PavelPll/RNA_transformer**

# OBJECTIVES

1. Generate hidden representations of RNA molecules using modern Natural Language Processing (NLP) approaches

2. Try to use these representations to describe some biological processes

# The idea of transformers

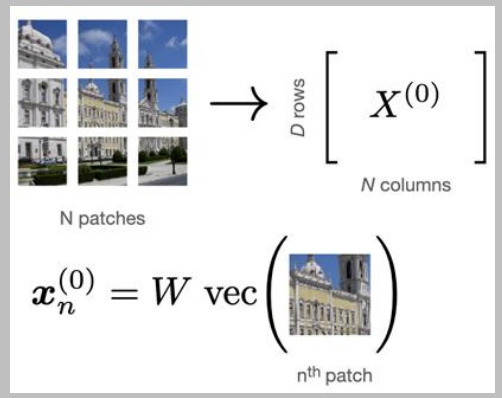
Embedding Layer

$N$ tokens

$$X^{(0)} = \left( \boldsymbol{x}_0^{(0)} \quad \dots \quad \boldsymbol{x}_n^{(0)} \quad \dots \quad \boldsymbol{x}_N^{(0)} \right) \Big\} D$$

## Encoding an image

$\boldsymbol{x}_n^{(0)}$ is a token




**vec operator**: Each patch is reshaped into a vector by the vec operator.
**matrix $W$** : maps a vector (the patch) to a D dimensional vector $\boldsymbol{x}_n^{(0)}$.
*[Dosovitskiy et al., 2021]*

## Iteratively applying a transformer block

hidden states

$$X^{(m-1)} = \left( \boldsymbol{x}_0^{(m-1)} \quad \dots \quad \boldsymbol{x}_n^{(m-1)} \quad \dots \quad \boldsymbol{x}_N^{(m-1)} \right) \rightarrow X^{(m)} = \left( \boldsymbol{x}_0^{(m)} \quad \dots \quad \boldsymbol{x}_n^{(m)} \quad \dots \quad \boldsymbol{x}_N^{(m)} \right)$$

hidden states

$$\boldsymbol{y}_n^{(m)} = \sum_{n'=1}^{N} x_{n'}^{(m-1)} A_{n',n}^{(m)} \quad \rightarrow \quad \sum_{h=1}^{H} V_h^{(m)} X^{(m-1)} A_h^{(m)}$$

$$Y^{(m)} = \left( \boldsymbol{y}_0^{(m)} \quad \dots \quad \boldsymbol{y}_n^{(m)} \quad \dots \quad \boldsymbol{y}_N^{(m)} \right) \Rightarrow \boldsymbol{x}_n^{(m)} = MLP_\theta \left( \boldsymbol{y}_n^{(m)} \right)$$

the final layer $M$
sequence_embedding

$$h = \sum_{n=1}^{N} \boldsymbol{x}_n^{(M)}$$

## The attention matrix

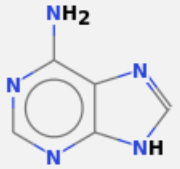
$$A_{n,n'} = \frac{x_n^T x_{n'}}{\sum_{n''=1}^{N} x_{n''}^T x_{n'}} \rightarrow \frac{\exp\left(x_n^T x_{n'}\right)}{\sum_{n''=1}^{N} \exp\left(x_{n''}^T x_{n'}\right)} \rightarrow \frac{\exp\left(x_n^T \boldsymbol{U}^T \boldsymbol{U} x_{n'}\right)}{\sum_{n''=1}^{N} \exp\left(x_{n''}^T \boldsymbol{U}^T \boldsymbol{U} x_{n'}\right)} \rightarrow \frac{\exp\left(x_n^T \boldsymbol{U}_k^T \boldsymbol{U}_q x_{n'}\right)}{\sum_{n''=1}^{N} \exp\left(x_{n''}^T \boldsymbol{U}_k^T \boldsymbol{U}_q x_{n'}\right)}$$

$$A_{n,n'} = \frac{\exp\left(k_n^T q_{n'}\right)}{\sum_{n''=1}^{N} \exp\left(k_{n''}^T q_{n'}\right)}, \quad \text{where} \quad \begin{cases} q_{h,n}^{(m)} = U_q^{(m)} x_n^{(m-1)}, & \text{queries} \\ \boldsymbol{k}_{h,n}^{(m)} = \boldsymbol{U}_k^{(m)} \boldsymbol{x}_n^{(m-1)}, & \boldsymbol{keys} \end{cases}$$

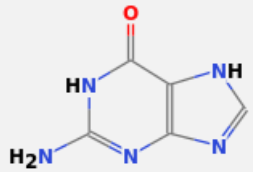
*[Turner R.E., https://arxiv.org/abs/2304.10557, 2023]*

# What is RNA molecule ?

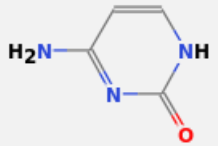
**A** = Adenine ($C_5H_5N_5$)



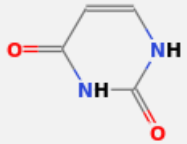
**G** = Guanine ($C_5H_5N_5O$)



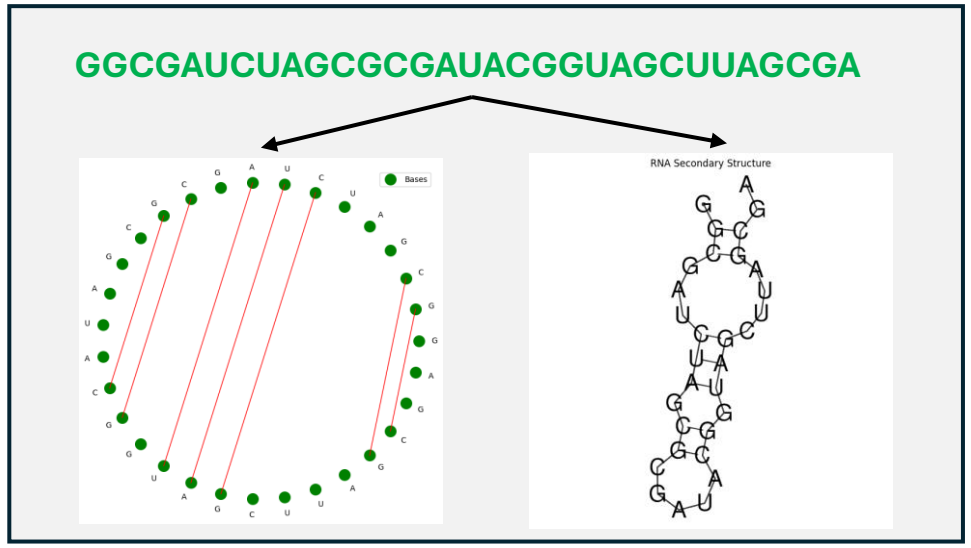
**C** = Cytosine ($C_4H_5N_3O$)

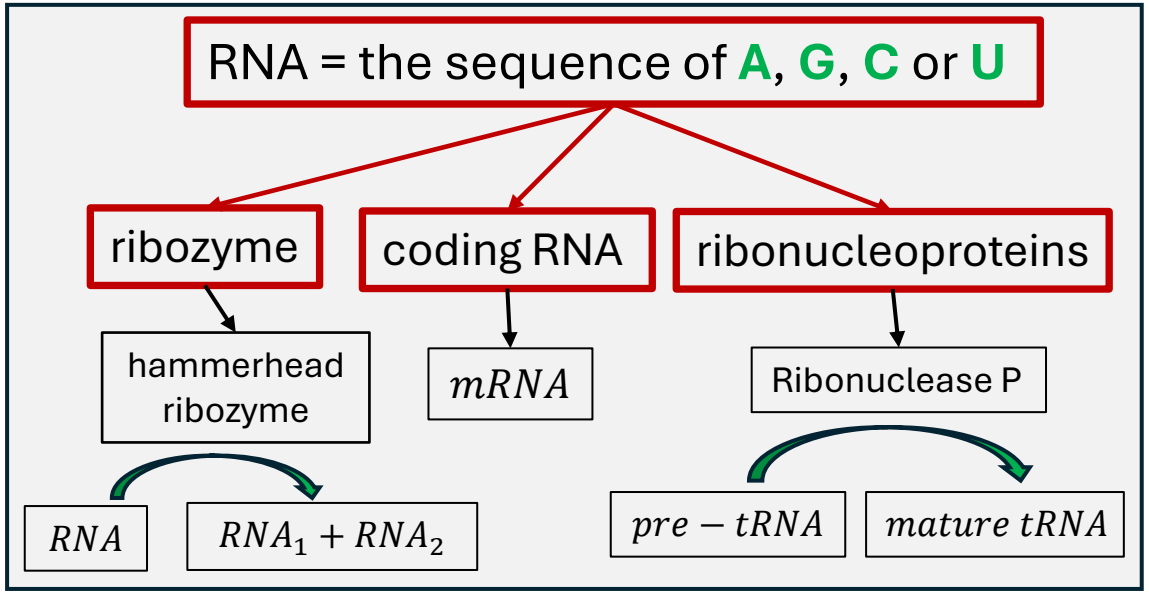


**U** = Uracil ($C_4H_4N_2O_2$)



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



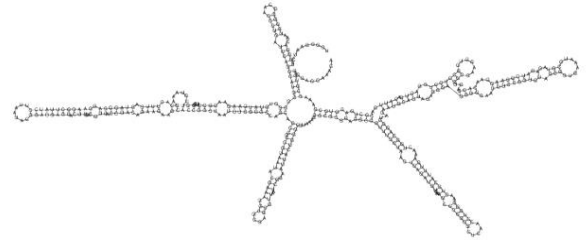



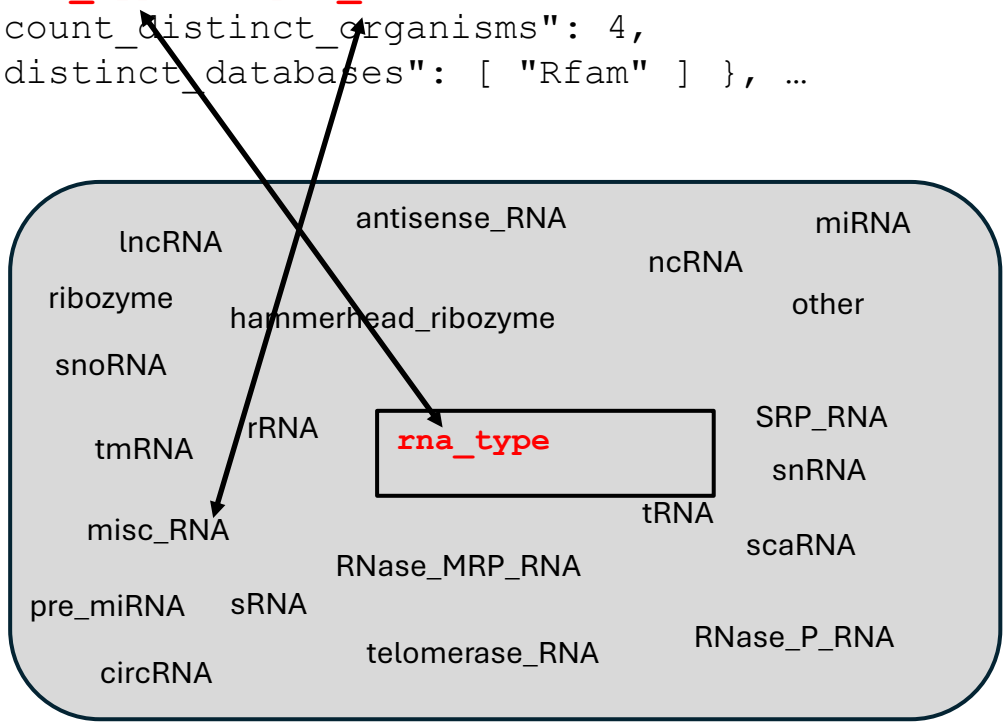
# The dataset: RNAcentral database

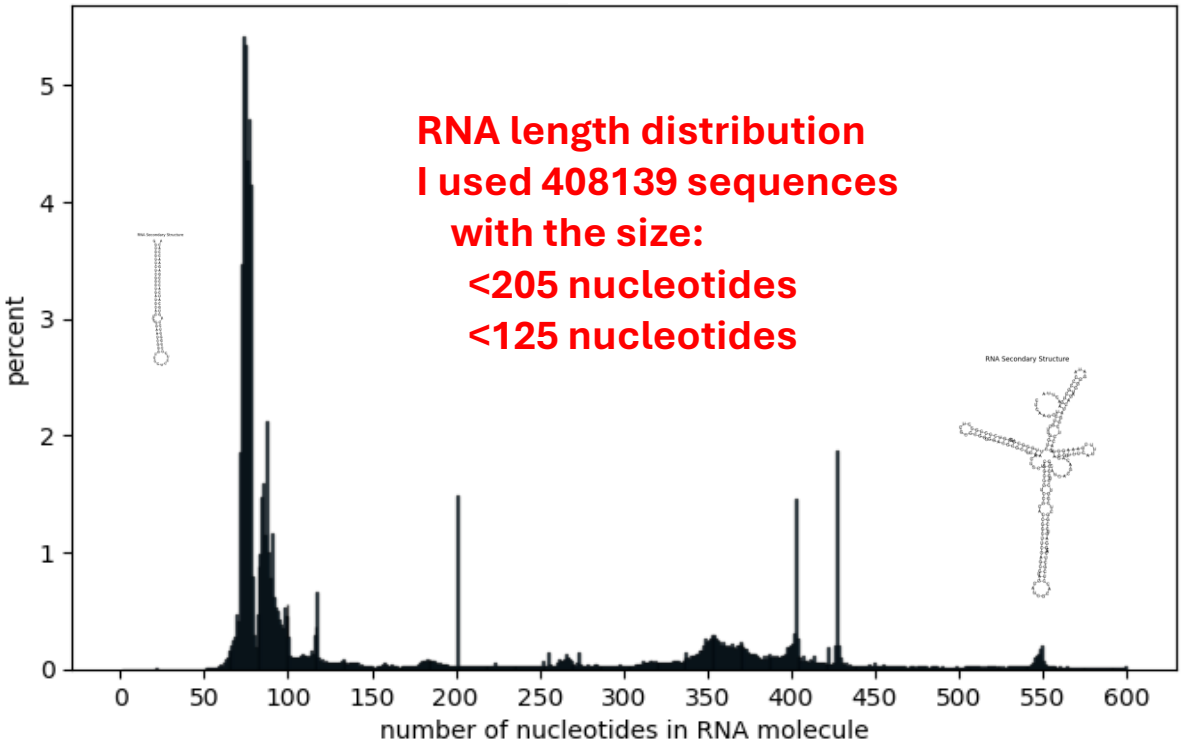
**RNAcentral Browsable API**

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 autoencoder (vanilla transformer)

AUGGAUGGUUGA

Vocab = {"U":6, "G":7, "C":8, "A":9,}

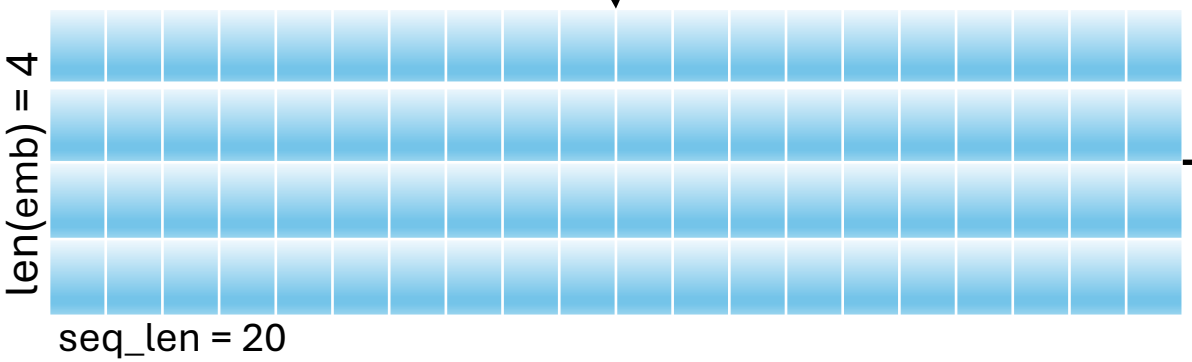
| 9 | 6 | 7 | 7 | 9 | 6 | 7 | 7 | 6 | 6 | 7 | 9 |
|---|---|---|---|---|---|---|---|---|---|---|---|

RNA_seq_len = 12

Vocab = { "START":1, « END":2, « PAD":3}

| 1 | 9 | 6 | 7 | 7 | 9 | 6 | 7 | 7 | 6 | 6 | 7 | 9 | 2 | 3 | 3 | 3 | 3 | 3 | 3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|

seq_len = 20




hidden representation

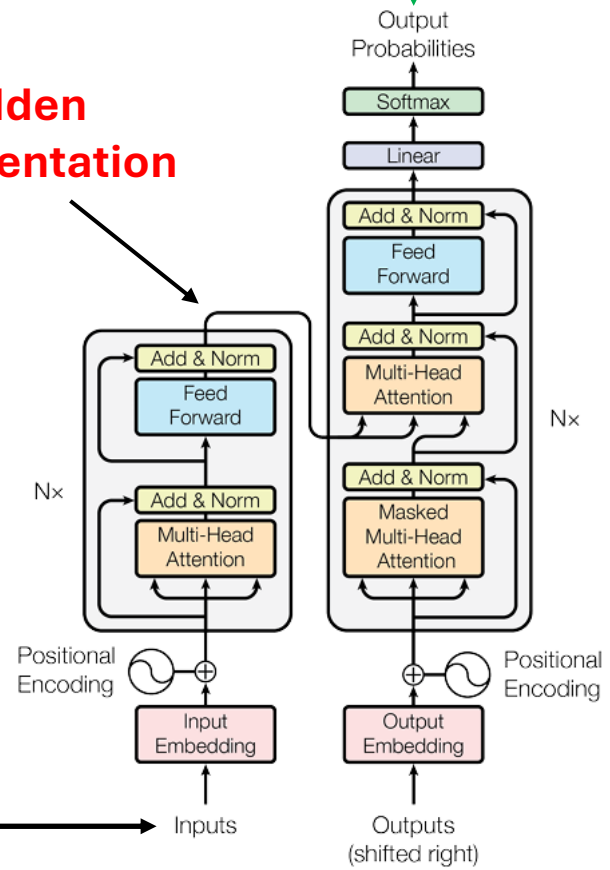
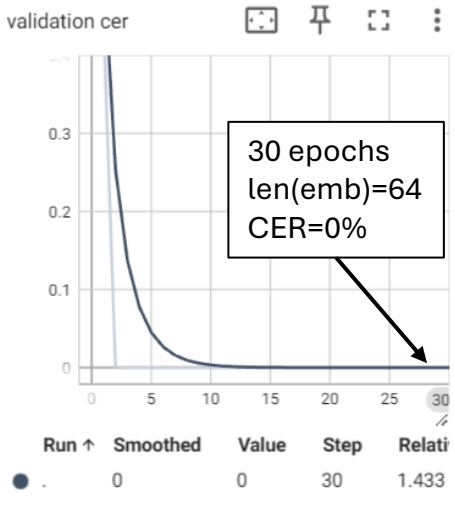
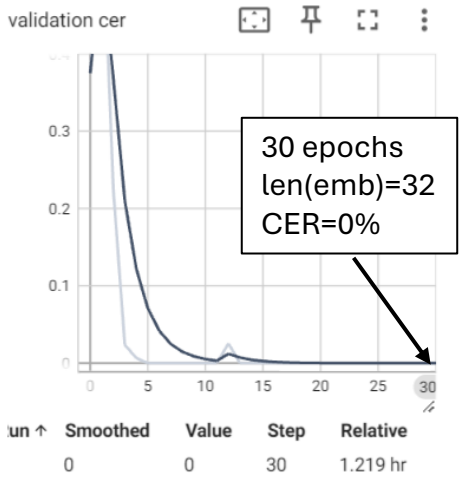


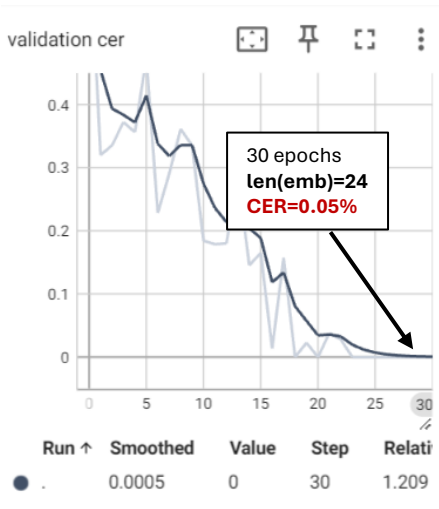

Figure 1: The Transformer - model architecture.

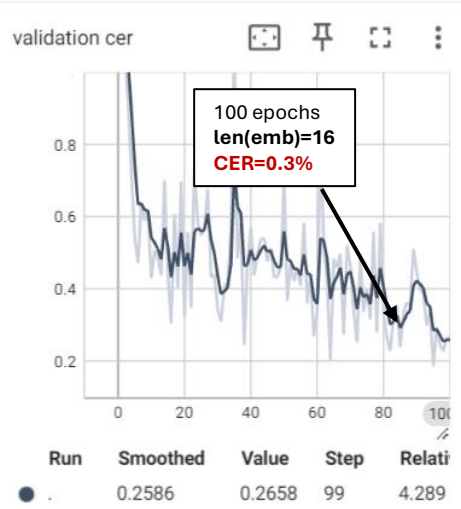
Ashish Vaswani et al., Attention Is All You Need
https://doi.org/10.48550/arXiv.1706.03762

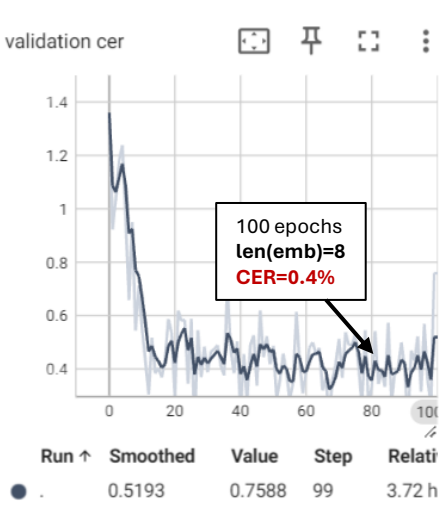
# RESULTS for RNA autoencoder validation




**len(emb)= 64**
**CER = 0%**

**len(emb)= 32**
**CER = 0%**

**len(emb)= 24**
**CER = 0.05%**

**len(emb)= 16**
**CER = 0.3%**

**len(emb)= 8**
**CER = 0.4%**

**CER (Character Error Rate) calculates the proportion of incorrect nucleotides (insertions, deletions, and substitutions) relative to the total number of nucleotides.**

**CER changes from 0% to 0.4% with decreasing the length of embeddings.**

# RNA classification (BERT-like transformer)

**AUGGAUGGUUGA**

Vocab = {"U":6, "G":7, "C":8, "A":9,}

| 9 | 6 | 7 | 7 | 9 | 6 | 7 | 7 | 6 | 6 | 7 | 9 |
|---|---|---|---|---|---|---|---|---|---|---|---|

RNA_seq_len = 12

Vocab = { "START":1, « END":2, « PAD":3}

| 1 | 9 | 6 | 7 | 7 | 9 | 6 | 7 | 7 | 6 | 6 | 7 | 9 | 2 | 3 | 3 | 3 | 3 | 3 | 3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|

seq_len = 20

len(emb) = 4

seq_len = 20

lncRNA, antisense_RNA, miRNA, ncRNA, ribozyme, hammerhead_ribozyme, other, snoRNA, tmRNA, rRNA, **rna_type**, SRP_RNA, snRNA, misc_RNA, tRNA, RNase_MRP_RNA, scaRNA, pre_miRNA, sRNA, RNase_P_RNA, telomerase_RNA, circRNA

**classification HEAD**

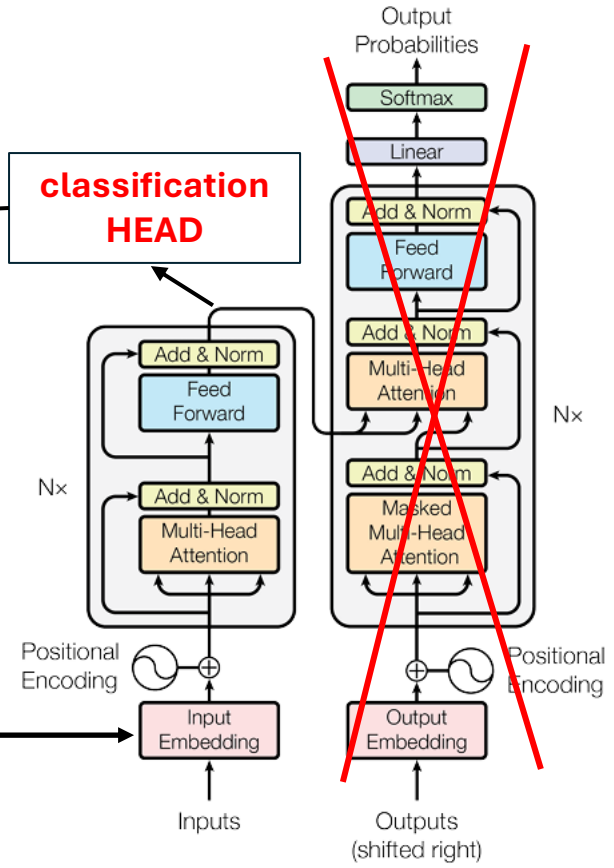
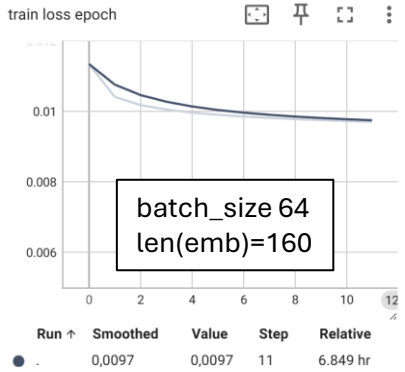
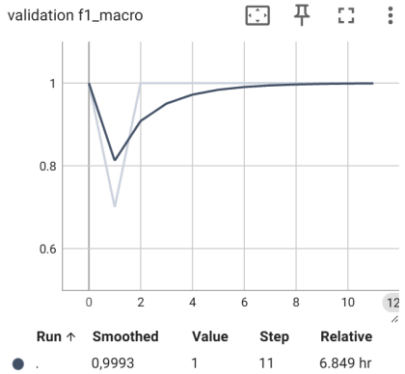
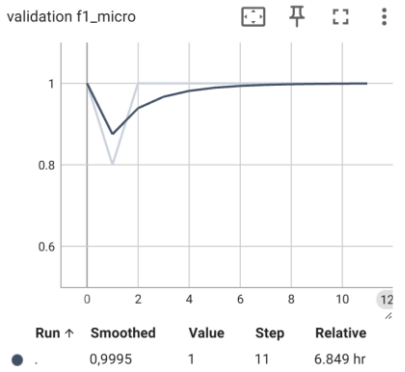



Figure 1: The Transformer - model architecture.

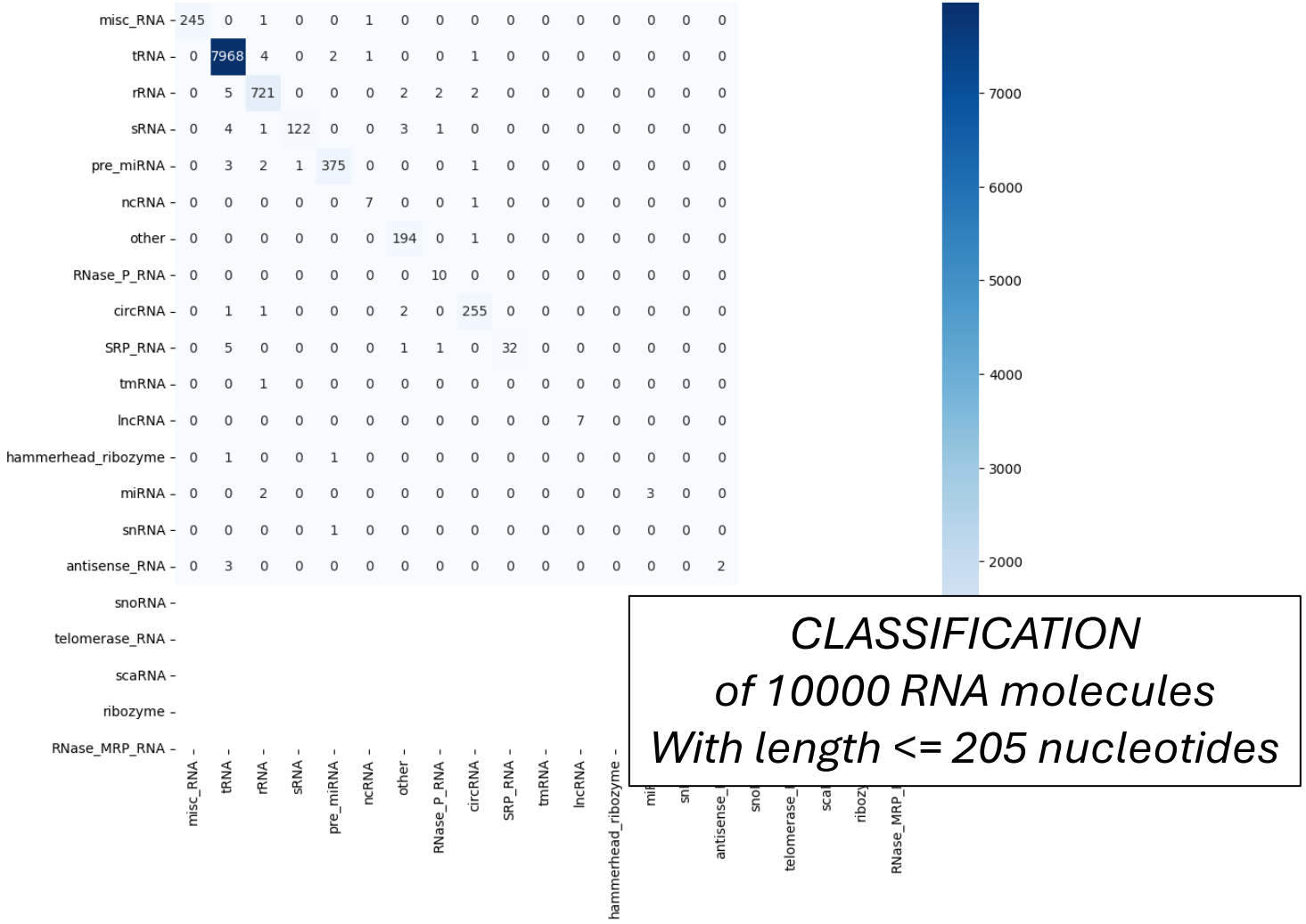
# RESULTS for RNAclassificator validation




VALIDATION
f1_micro 0.9941
f1_macro 0.7351456585589439




*CLASSIFICATION*
*of 10000 RNA molecules*
*With length <= 205 nucleotides*

# RNA autoencoder (BERT-like transformer)

AUGGAUGGUUGA

Vocab = {"U":6, "G":7, "C":8, "A":9,}

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|

seq_len = 20

seq_len = 20

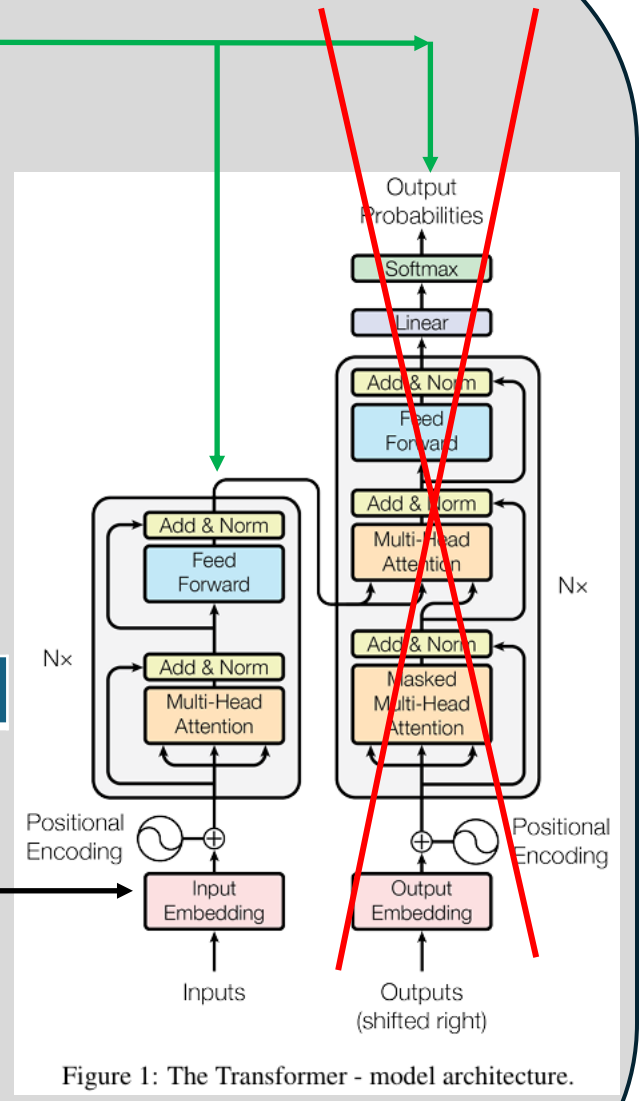
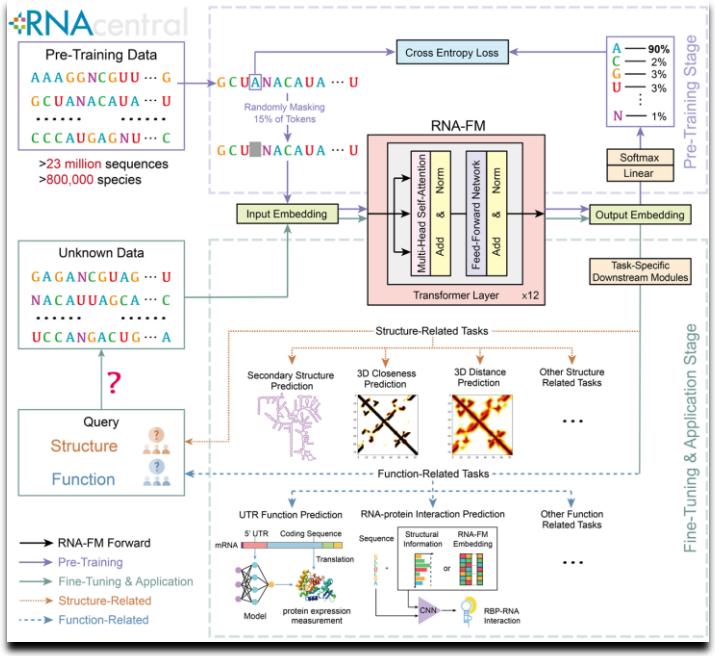



Figure 1: The Transformer - model architecture.

## STATE of the ART RNAFM model

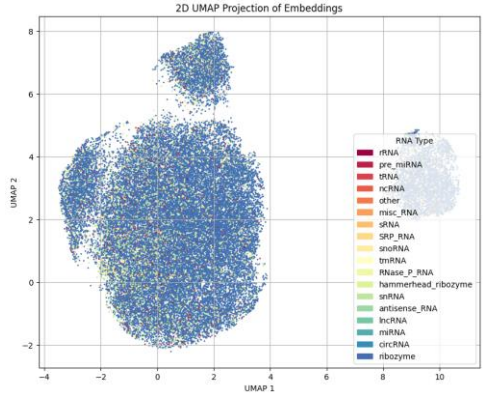



J.Chen et al.,
https://www.biorxiv.org/content/10.1101/2022.08.06.503062v2

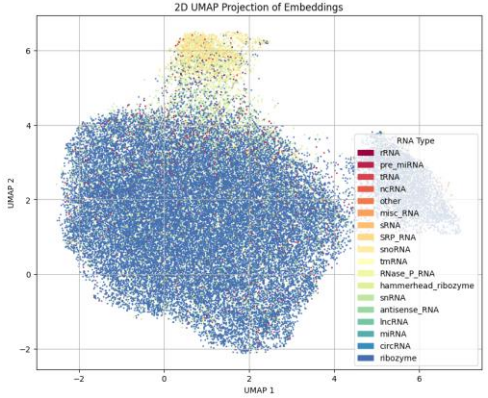
# RESULTS for RNA autoencoder validation (for 40 000 RNAs)

- *secondary/3D structure prediction*
- *SARS-CoV-2 genome structure and evolution prediction*
- *protein-RNA binding preference modeling*
- *gene expression regulation modeling*

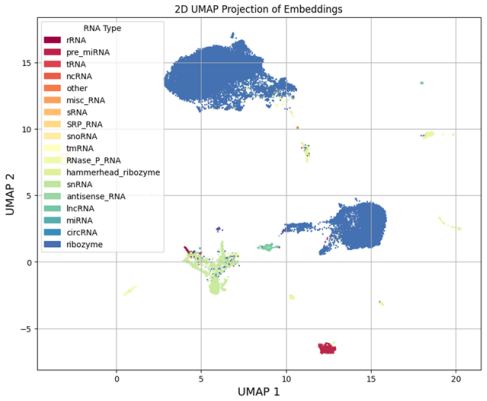
reconstruction loss (1)
random embedding
batch_size = 64,
len(emb) = 64




reconstruction loss (1)
**custom embedding**
batch_size = 64,
len(emb) = 64

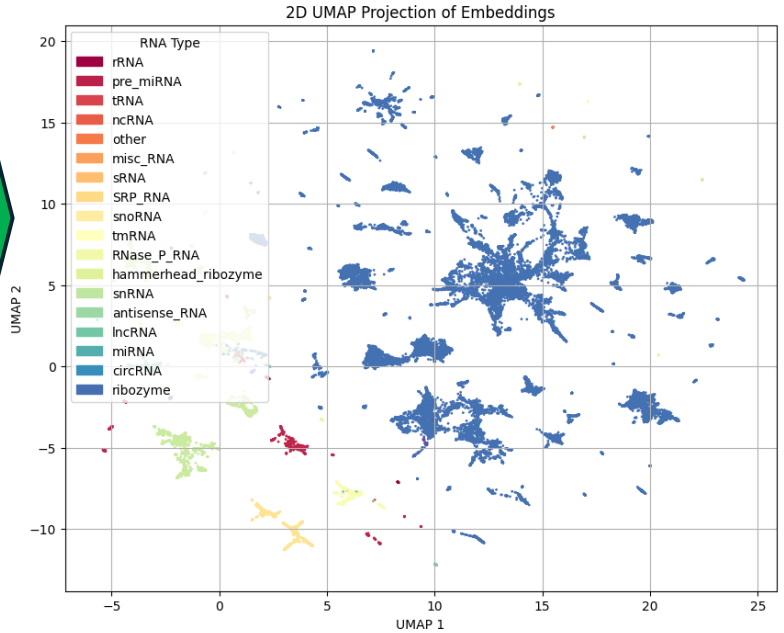



reconstruction loss (0.9)
**classification loss (0.1)**
custom embedding
batch_size = 64
len(emb) = 160




4 vanilla transformer-based encoder blocks
400 000 sequences
L × 64 embedding matrix for each RNA (length L)
RTX 4060, 8GB for 12 hours

**STATE of the ART**
**RNAFM model**



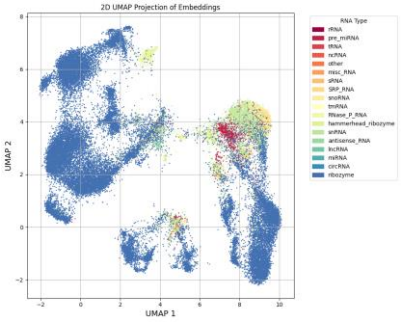

12 transformer-based bidirectional encoder blocks
23 million sequences
L × 640 embedding matrix for each RNA (length L)
eight A100 GPUs of 80 GB memories for one month

IMPROVEMENT direction

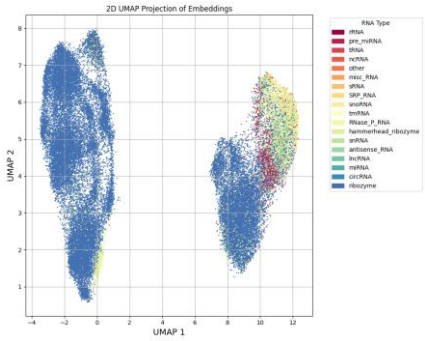
# Further Improvement (for 40 000 RNAs)

vanilla transformer-based

reconstruction loss (1)
random embedding
len(emb) = 160

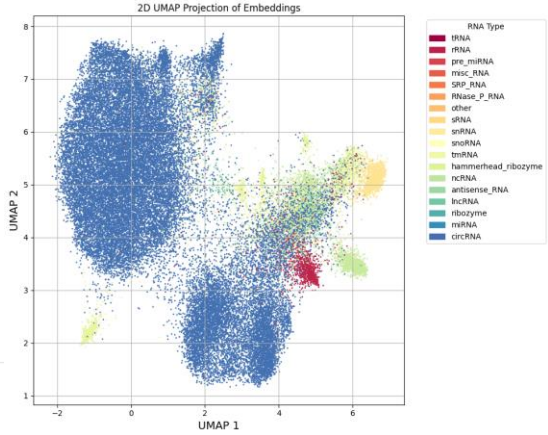



reconstruction loss (1)
**custom embedding**
len(emb) = 160



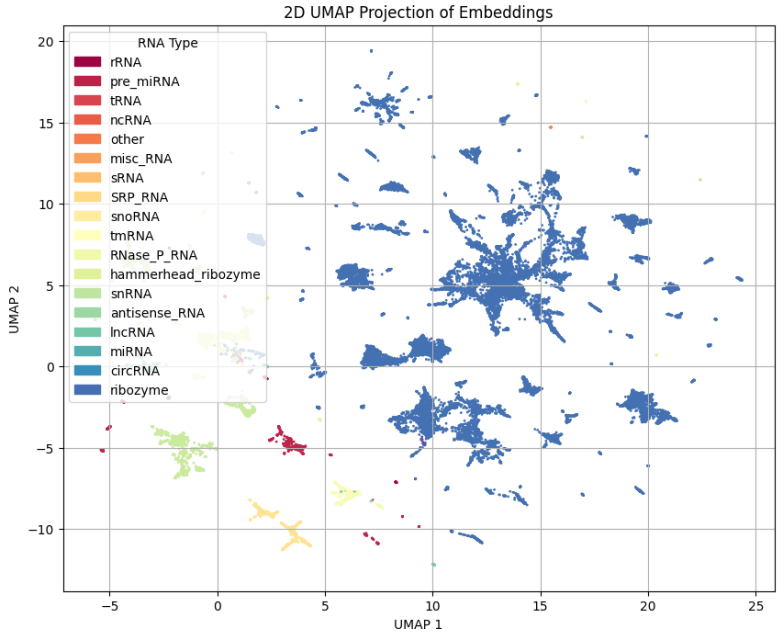

**0.4 million sequences**
**4 blocks, 8 heads**
**L × 64, 160 embedding matrix for each RNA**
**length Lmax = 205 nucleotides**
**RTX 4060, 8GB for 12 hours**

Composite autoencoder

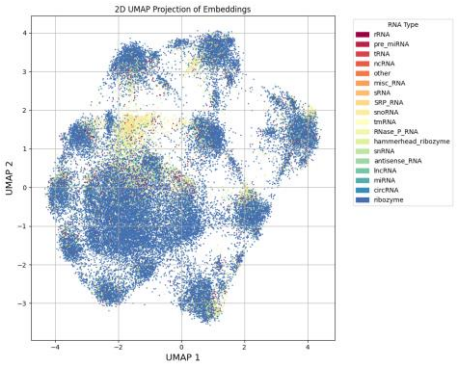


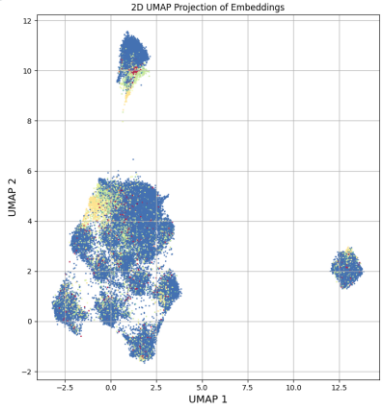

- *secondary/3D structure prediction*
- *SARS-CoV-2 genome structure and evolution prediction*
- *protein-RNA binding preference modeling*
- *gene expression regulation modeling*

**STATE of the ART**
**RNAFM model**




BERT-like transformer




**IMPROVEMENT direction**

**23 million sequences**
**12 blocks, 16 heads**
**L × 640 embedding matrix for each RNA (length L)**
**eight A100 GPUs of 80 GB memories for one month**

# Word embeddings arithmetic

## ENGLISH language

Laptop ≈ Computer + Portable
Smartphone ≈ Phone + Smart

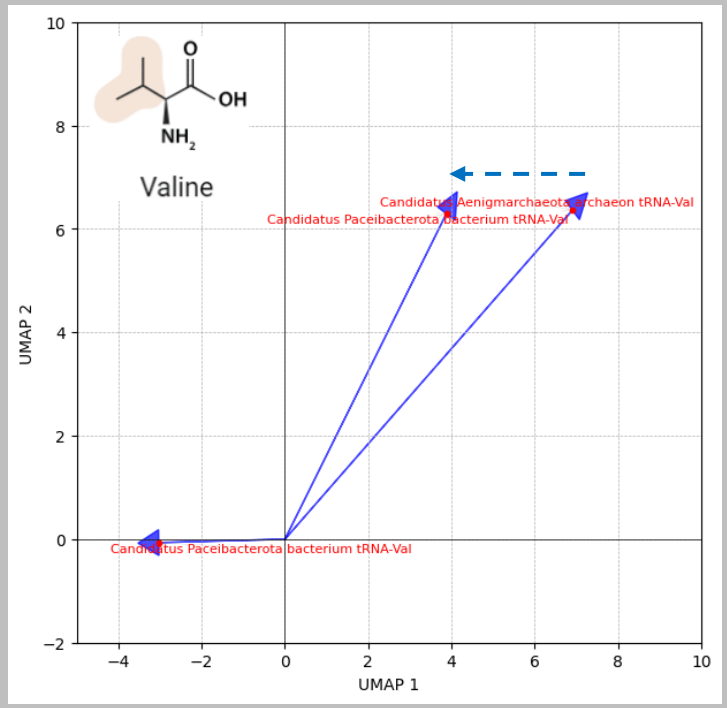
Fast + More ≈ Faster
Happy + Not ≈ Sad

King – Man + Woman ≈ Queen
Paris – France + Italy ≈ Rome

Amino acids from:
https://www.rapidnovor.com/structure-of-an-amino-acid/

## RNA language

**archaea and bacteria often coexist in various environments**




Candidatus Paceibacterota bacterium **tRNA-Val**

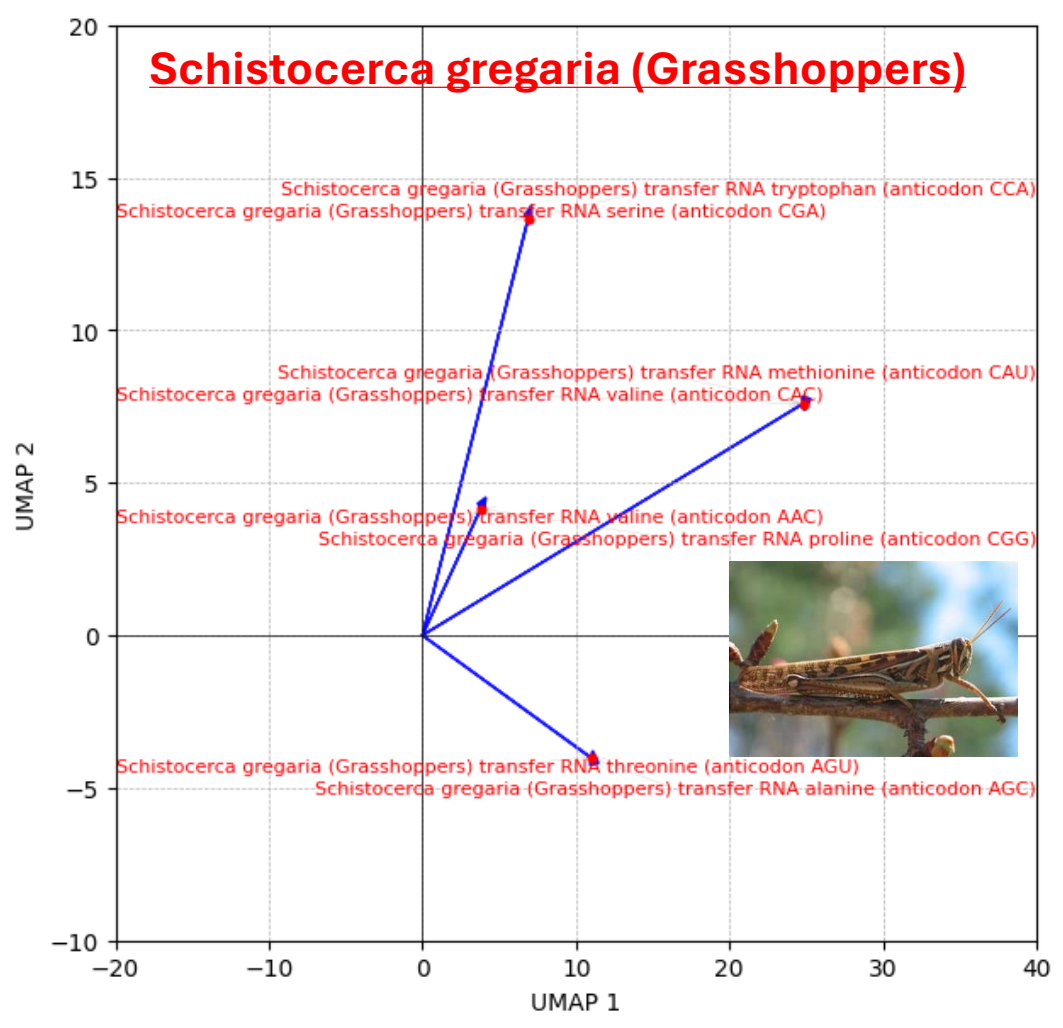
Candidatus Paceibacterota bacterium **tRNA-Val**

tRNA(73nt) = tRNA(73nt) + tRNA(74nt)

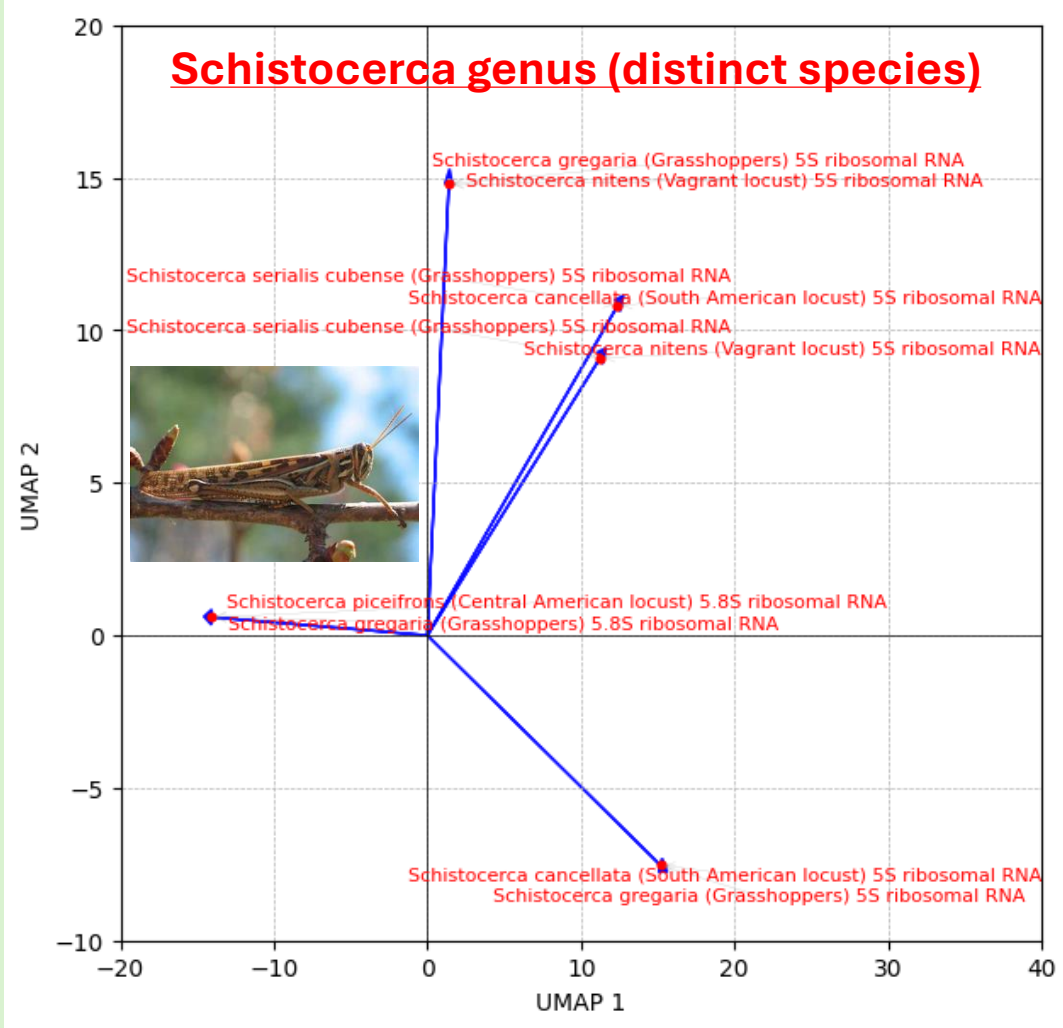
Candidatus Aenigmarchaeota archaeon **tRNA-Val**

# Word arithmetic: tRNA and rRNA of Schistocerca

## Transfer RNA (tRNA)



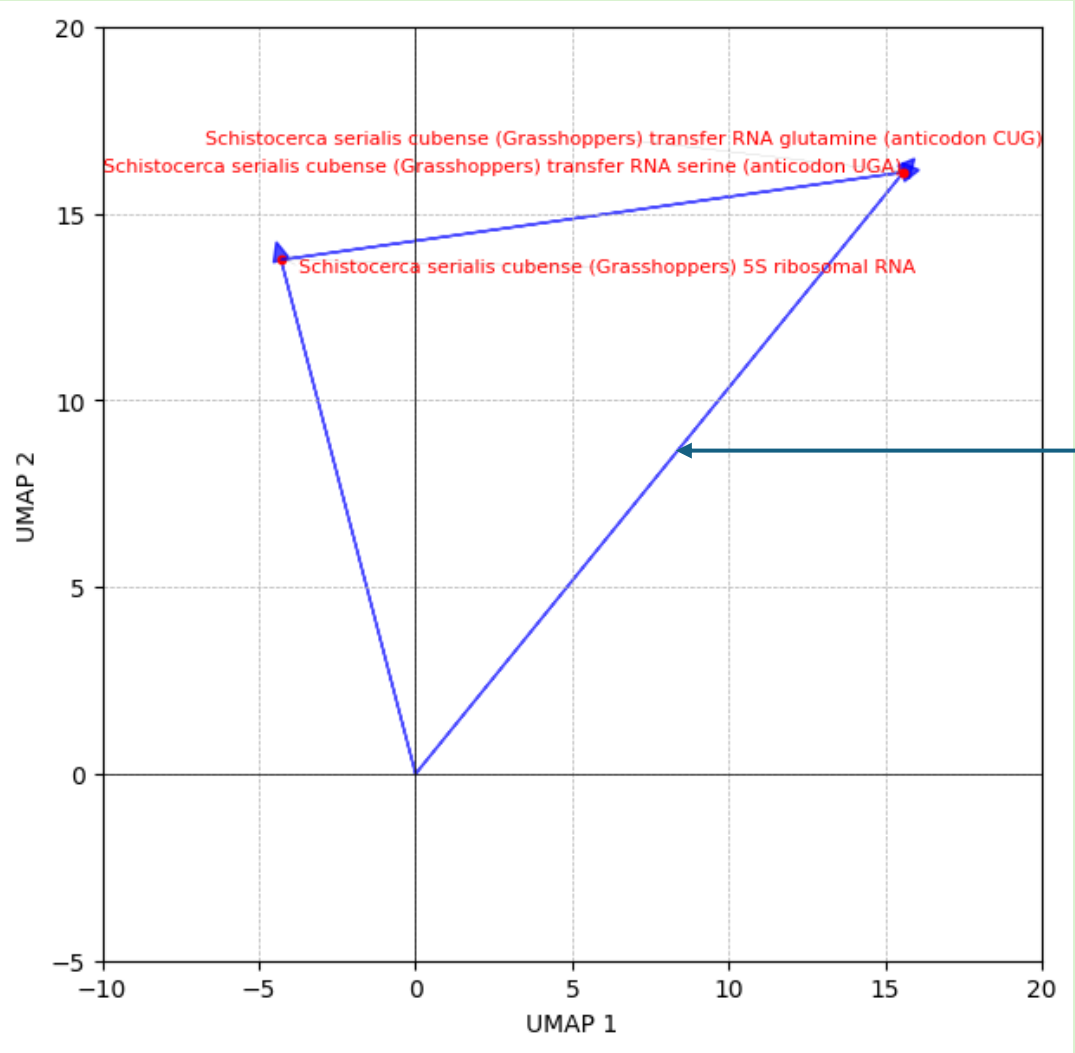

## 5S Ribosomial RNA (rRNA)




**Each arrow consists of two sub-arrows to identify two similar RNAs that share the same hidden representation.**

The image of Schistocerca from here: https://th.bing.com/th/id/OIP.Nzhi77J6_VosvgaRUmvWzAAAAA?rs=1&pid=ImgDetMain

# Schistocerca serialis cubense (word embedding arithmetic)




Schistocerca serialis cubense
**5S ribosomal RNA**

tRNA(72nt) = **rRNA**(121nt) + tRNA(75nt)

transfer RNA glutamine (anticodon CUG)

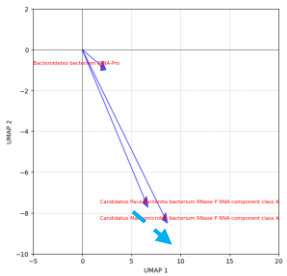
transfer RNA serine (anticodon UGA)

Schistocerca image from: https://th.bing.com/th/id/OIP.Nzhi77J6_VosvgaRUmvWzAAAAA?rs=1&pid=ImgDetMain

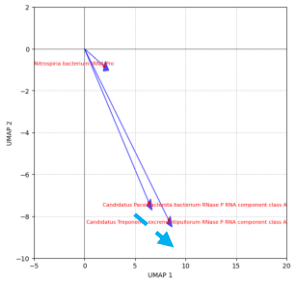
# Dependence of tRNA on other ncRNAs

| ncRNA Type | Regulates tRNA-modifying enzyme genes at the genomic level. | tRNA-Modifying Enzymes Physically Interacts with tRNA-Modifying Enzymes |
|---|---|---|
| lncRNA | **Most versatile**—acts **before or after** mRNA is made, affects transcription, translation, or protein function. | Yes |
| miRNA | Regulate gene expression **post-transcriptionally** (after mRNA is made) | No direct interaction |
| circRNA | **binds to miRNAs**, preventing them from regulating **tRNA-modifying enzymes**. | No direct interaction |
| sRNA | Bacterial equivalent of miRNA, regulates **post-transcriptionally** but without a RISC complex. | No direct interaction |
| snoRNA | Does not regulate tRNA gene expression | snoRNA binds to a **tRNA-modifying enzyme** (e.g., methyltransferase or pseudouridine synthase). |
| **RNase P RNA** | Does not regulate tRNA gene expression | Cleaves the **5' leader sequence**, generating the mature **5' end** of tRNA, allowing **CCA addition** at the **3' end** and amino acid charging. |

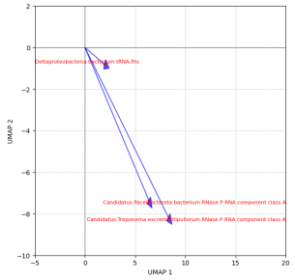
-RNase_P_RNA(387nc)+ RNase_P_RNA(**388nc**)+ tRNA(74nc) = 0



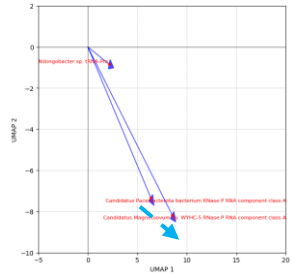
-tRNA(77nc)+ RNase_P_RNA(**413nc**)- RNase_P_RNA(**362nc**) = 0



-tRNA(74nc)- RNase_P_RNA(**388nc**)+ RNase_P_RNA(**413nc**) = 0

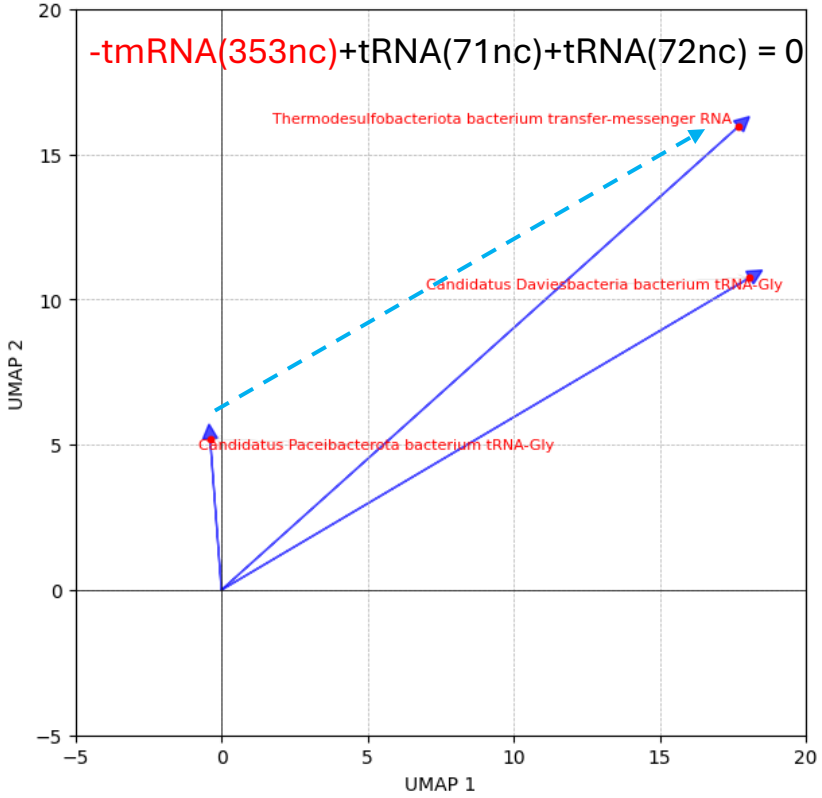


-RNase_P_RNA(406nc)+ tRNA(77nc)+ RNase_P_RNA(**362nc**) = 0

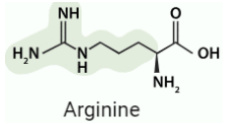


Two **RNase P RNA** with tRNA of proline

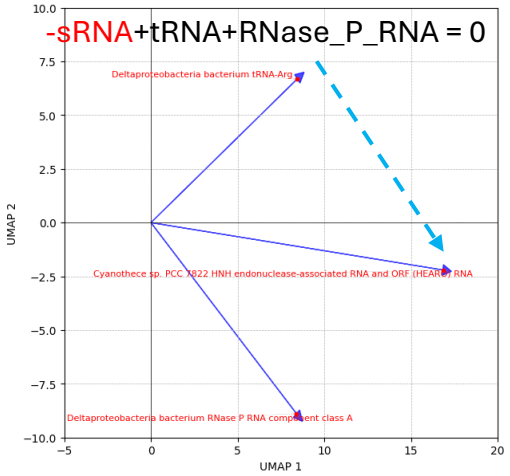
**tmRNA (Transfer-Messenger RNA)**
**Rescues stalled ribosomes** when an mRNA lacks a stop codon.
Acts as both **tRNA and mRNA**.
It is charged with **alanine (Ala)**.



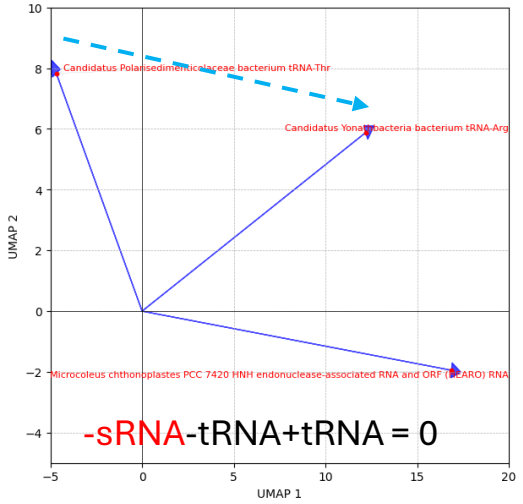
# Cyanobacteria and tRNA of Arginine (3*N* atoms)



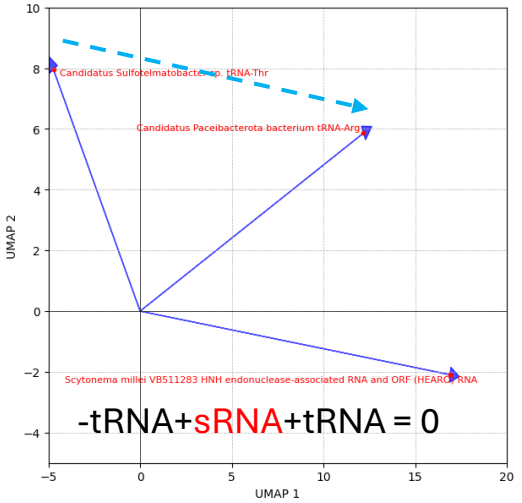

## Cyanothece sp. PCC 7822




## Microcoleus chthonoplastes PCC 7420




## Scytonema millei VB511283

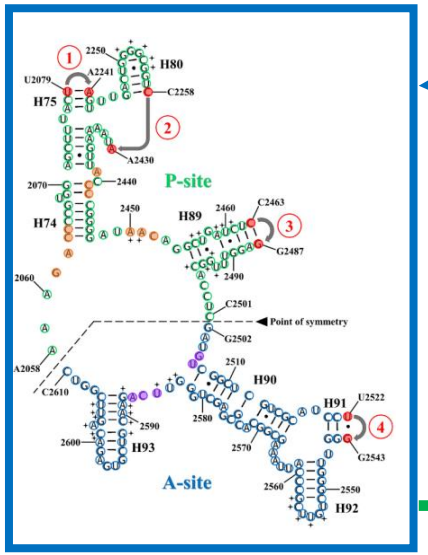



| Organism Type | Estimated Origin (B years) | Related Events |
|---|---|---|
| **LUCA (Last Universal Common Ancestor)** *(Early prokaryotic ancestor of all life)* | ~3.5–3.8 | First known life on Earth, anaerobic metabolism, no oxygen in atmosphere |
| First Cyanobacteria (Ancestors of **Cyanothece sp. PCC 7822**) | ~2.7 | Oxygenic photosynthesis begins, starts producing atmospheric oxygen |
| Great Oxygenation Event (GOE) | ~2.5 | Oxygen starts accumulating in atmosphere, first mass extinction of anaerobes |
| Filamentous Cyanobacteria (Ancestors of **Microcoleus chthonoplastes PCC 7420**) | ~2.0 | Stromatolites become widespread, more advanced cyanobacteria evolve |
| Heterocyst-forming Cyanobacteria (Ancestors of **Scytonema millei VB511283**) | ~1.5 | More efficient nitrogen fixation, evolution of heterocysts |

**Cyanobacteria** are among the oldest known life forms on Earth. **Cyanobacterium converts $N_2$ (dinitrogen) into $NH_3$.** This process, known as **biological nitrogen fixation**, allows to assimilate nitrogen for building essential biomolecules

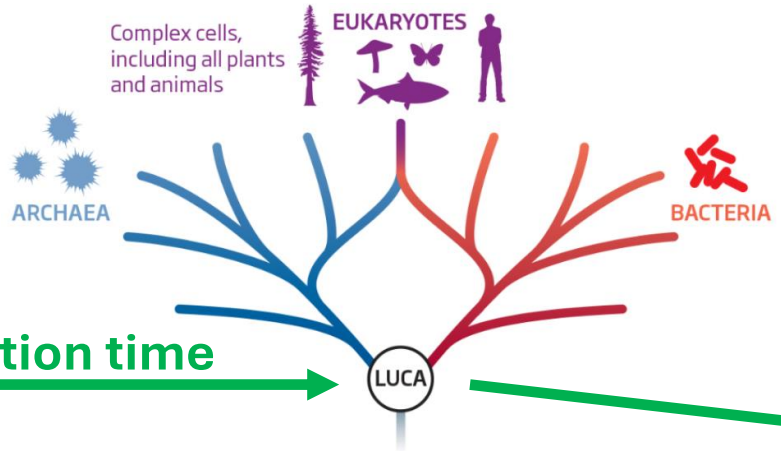
$$N_2 + 8H^+ + 8e^- + 16ATP = 2NH_3 + H_2 + 16ADP + 16P_i$$

# rRNA evolution: Age Variability Across Different Regions




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*)

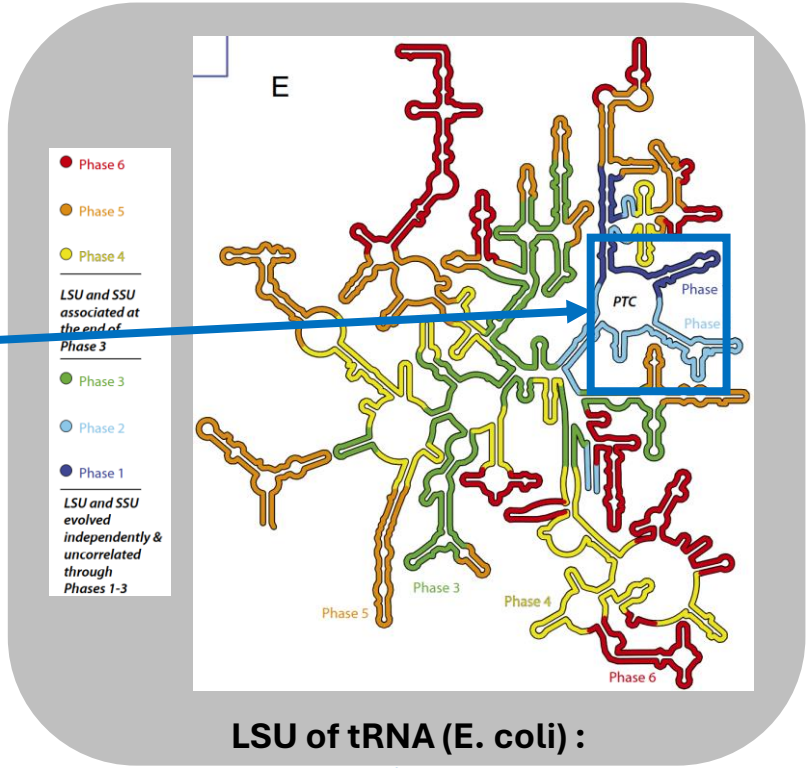
**the OLDEST functional part: always the same!**



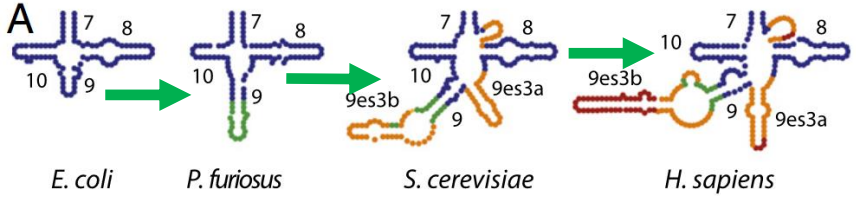

**evolution time**

**Last Universal Common Ancestor (LUCA)**

Image from:
https://www.pulseheadlines.com/earths-universal-common-ancestor-volcanic-origins/43890/




**LSU of tRNA (E. coli) :**
**Blue part is the oldest one**




Molecular level chronology of the evolution of the large ribosomal subunit (LSU) rRNA. Each accretion step adds to previous rRNA but leaves the underlying **core unperturbed** (Anton S. Petrov et al., PNAS, 2015)

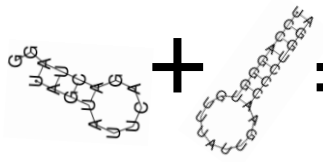
**Peptidyl Transferase Center (PTC) is the oldest part of ribosomes.**

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**.

# Peptidyl Transferase Center (PTC) Sequences

## the idea

the dimerization of two similar RNA structures

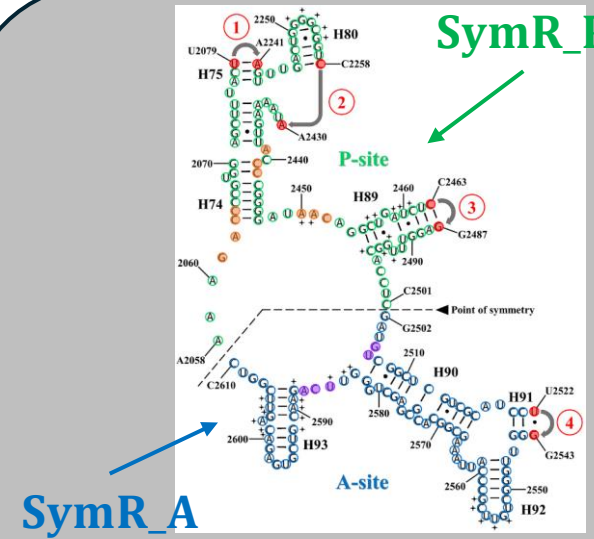


**PTC**

"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

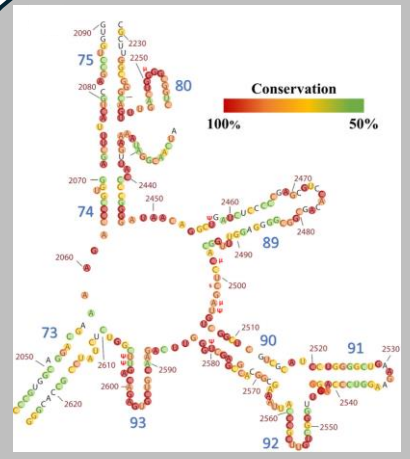
## pseudosymmetrical region




**SymR_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**

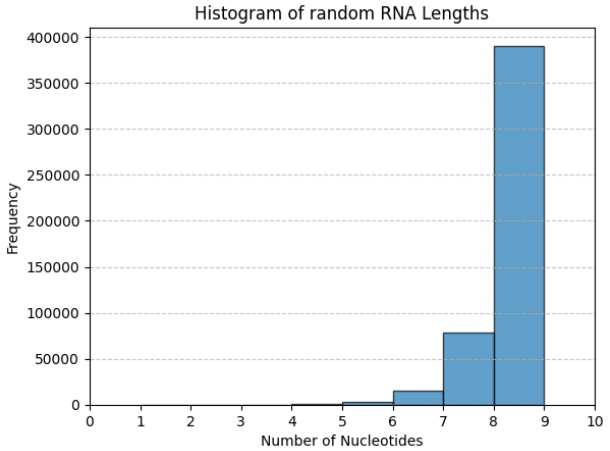
**Nucleotide CONSERVATION level:**
Red circles: 100% conservation (78 nt).
Orange circles: 90 to 99.9% conservation (68 nt)
Yellow circles: 70 to 89.9% (52nt)
Green circles: 50 to 69.9% conservation (49nt)
Black letters: less than 50% conservation (35nt)

(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

# Transformer Hidden representation of the oldest part (PTC) in two different basis sets

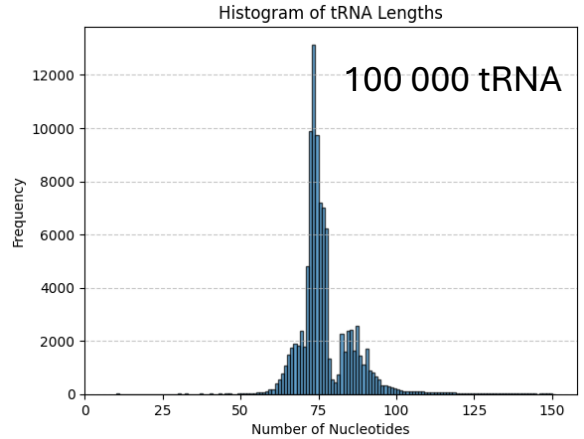
## BASIS I
## 488280 random RNAs
## With length <=8ncl



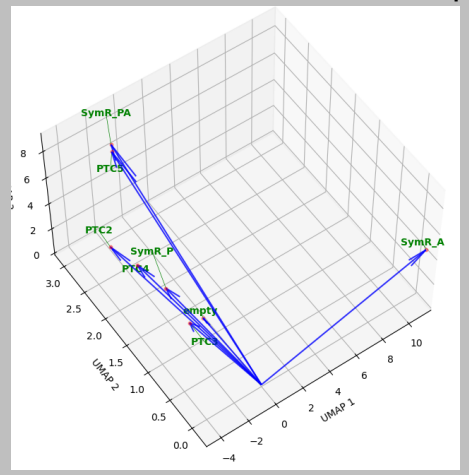

Peptidyl Transferase Center (**PTC**) is the oldest part of ribosomes.
PTC2: 549nc = 78nc(defined) + 471nc(undefined)
PTC3: 564nc = 146nc(defined) + 418nc(undefined)
PTC4: 583nc = 198nc(defined) + 385nc(undefined)
PTC5: 583nc = 247nc(defined) + 336nc(undefined)

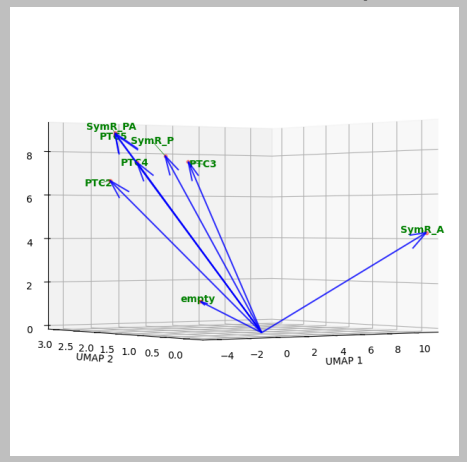
Pseudosymmetrical region (**SymR**; *Agmon et al., 2005*), derived from the LSU secondary structure of Thermus thermophilus
**SymR_A: 109nc = 89nc(defined) + 20nc(undefined)**
**SymR_P: 444nc = 89nc(defined) + 375nc(undefined)**
(*Petrov et al., 2013*).
(*Madhan R. Tirumalai et al., 2021*)

## BASIS II
## 100 000 tRNAs
## 50ncl <= length <= 125ncl




Visualization of the same 3D plot from two distinct viewpoints

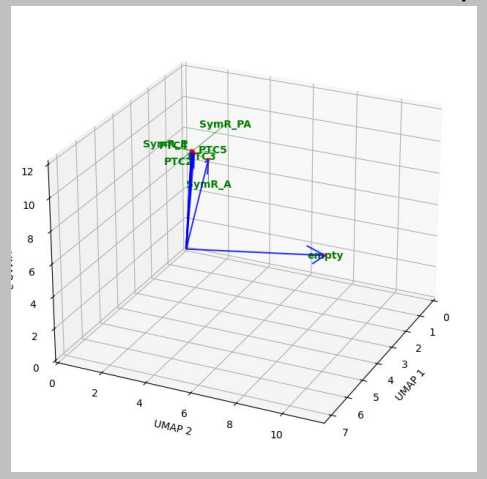


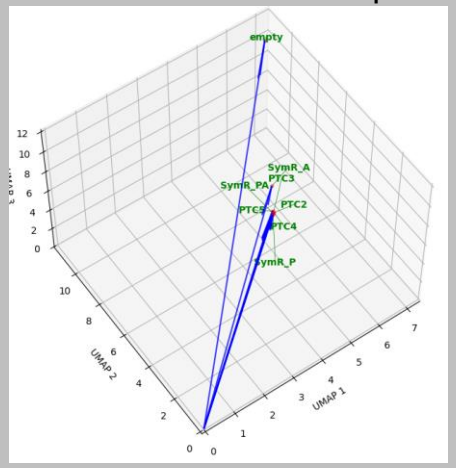




**SymR_A** ⊥ all

PTC5 = SymR_PA

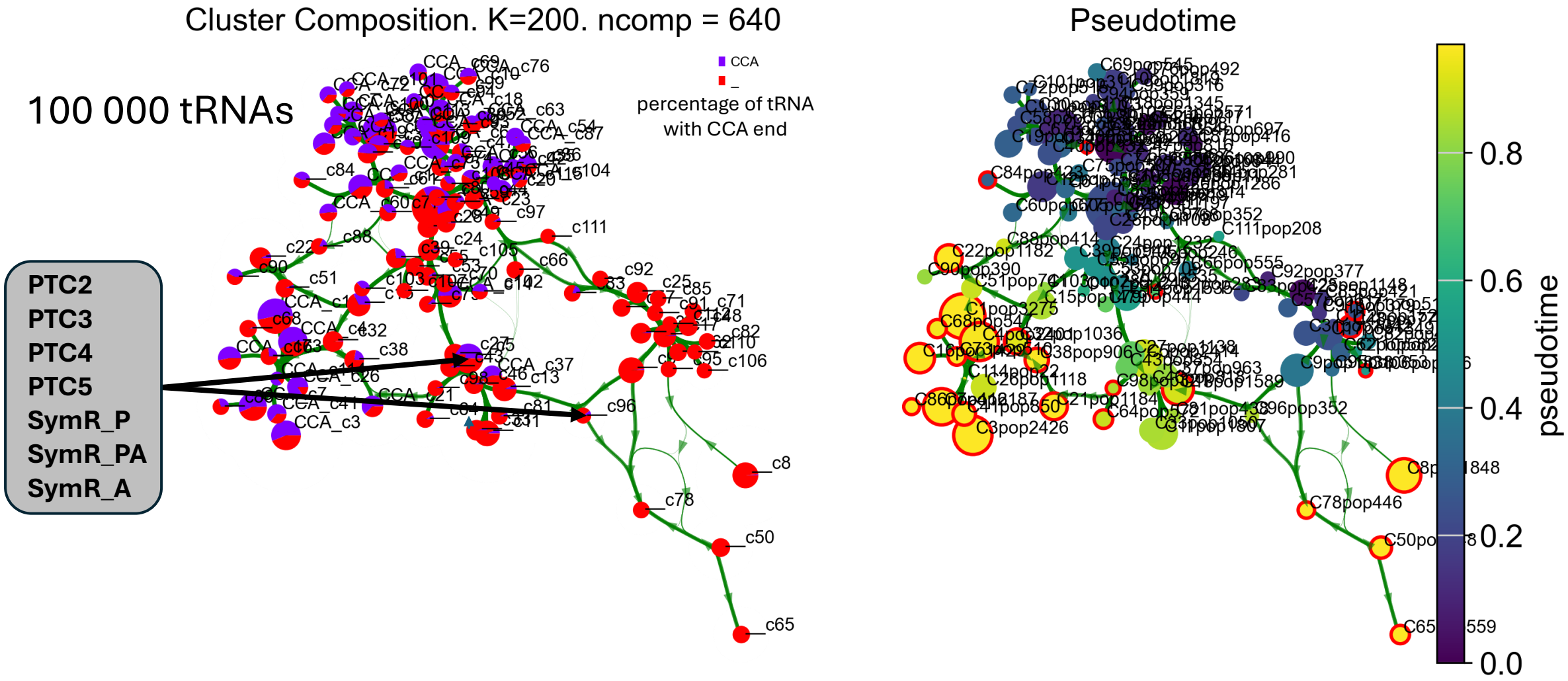
Visualization of the same 3D plot from two distinct viewpoints




**SymR_A** ≠ (PTC2 = PTC3 = PTC4 = PTC5 = SymR_PA = SymR_P)

# Single-Cell Inspired Analysis of tRNA and oldest rRNA

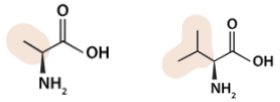



Peptidyl transferase center was detected in the 5th and 96th clusters.

Method used from : Generalized and scalable trajectory inference in single-cell omics data with VIA, Stassen et al., Nature Communications, 2021

# Single-Cell Inspired Analysis of tRNA and oldest rRNA multiple runs

two of the simplest and oldest amino acids.

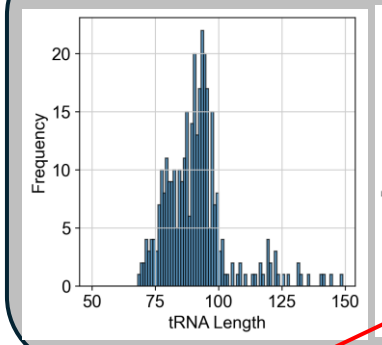


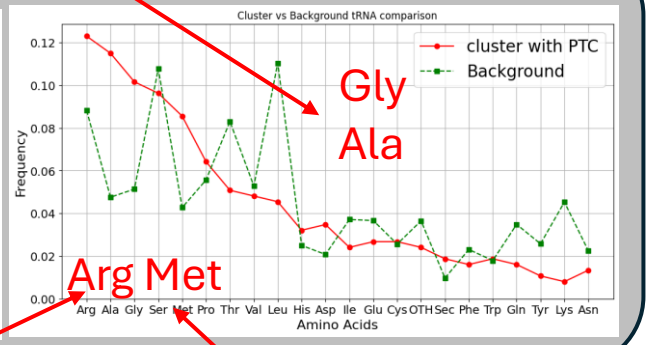
Alanine



Valine

Cluster96 with **PTC** (CCA=0.11, L=93.52nc):




Gly
Ala

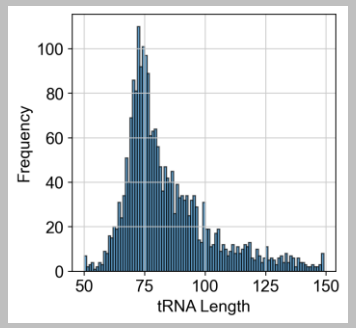
Arg Met

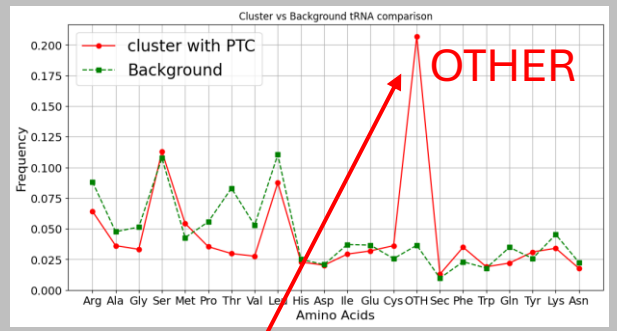
essential for stabilizing the negatively charged RNA phosphate backbone

encoded by the universal start codon

Four standard amino acids tRNA were detected above the background

Cluster5 with **PTC** (CCA=0.5,L= 97.15nc)

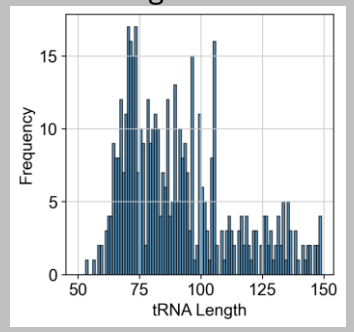



OTHER

other tRNA and pre-tRNA

OTHER (OTH)
aver length: 90.84nc




CCA percentage: 0.6

No standard amino acids tRNA were detected above the background

Peptidyl transferase center was detected in the 5th and/or 96th clusters.

Amino acid images from: https://www.rapidnovor.com/structure-of-an-amino-acid/

Method used from : Generalized and scalable trajectory inference in single-cell omics data with VIA, Stassen et al., Nature Communications, 2021

# CONCLUSIONS

- The vanilla transformer with custom embeddings and a masked training paradigm allows for deeper hidden representations than a BERT-like transformer

- Different ncRNAs are connected among each other by simple additive equations, some preliminary interpretations are proposed:
  - *Archaea and bacteria often coexist in various environments*
  - *Biological nitrogen fixation by Cyanobacteria*

- It is possible to compress the hidden representations of various PTCs into a single vector in 3D space by switching from a random basis to a tRNA basis

- The PTC is either associated with tRNA for Gly, Ala, Arg and Met, or with other pre-tRNAs not directly related to the standard 21 amino acids

PTC - Peptidyl Transferase Center of Ribosome

# What's Next?

1. **Refinement of RNA similarity measure:** Instead of using a single scalar (such as L2 distance), one can extract the individual attention head outputs before concatenation into the final embedding. This approach enables a more detailed characterization of RNA sequences, allowing for distinctions where two RNAs may be similar in one aspect but different in another.

2. **Refinement of autoencoder embeddings:** Use Graph Attention Network (GAT) to incorporate the graph structure and adjust the node (RNAs) representation based on the importance of its neighbors.