A Novel Approach to Understanding the Structural Basis of Ribosome Stalling by AMD1 and Cellular Arresting Peptides

Introduction

- Ribosome stalling is a process that regulates protein synthesis based on various factors, but dysregulated stalling can interrupt careful balances and influence **cancer progression**

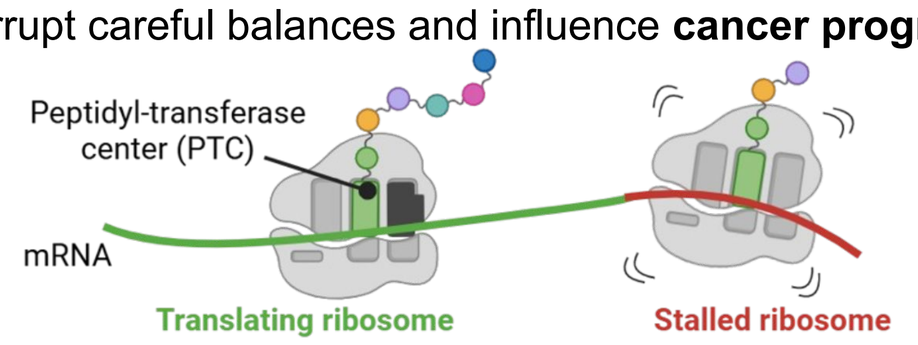


Fig 1. Translation and Stalling Overview. *Image created by finalist using BioRender, 2025*

- Adenosylmethionine decarboxylase 1 (**AMD1**) is an enzyme that helps make polyamines, essential to cell growth
- Prior research shows that its synthesis regulated by ribosomes pausing in the 5' and 3' mRNA untranslated regions (UTR), but exact mechanism in the 3' UTR is **unknown**

AMD1 is upregulated in prostate and breast cancers

- **Limited understanding** of AMD1 stalling **impedes current drugs** in trial, making it necessary to understand its stalling
- Current research methods, cryogenic electron microscopy and ribosome profiling, are **limited in accessibility**

1. **Stalled AMD1 C-peptide structure is unknown**
2. **Lack of computational analysis approaches**

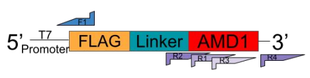
Methods

1. Elucidate the structure of the stalled AMD1 C-tail peptide

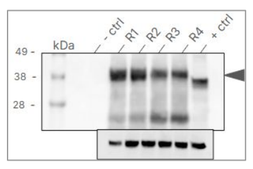
PCR + In vitro Transcription: Evaluate various reverse primers to generate stalled AMD1 C-tail

In vitro Translation: Evaluate primers, spermidine, and mRNA to improve ribosome nascent chain complexes

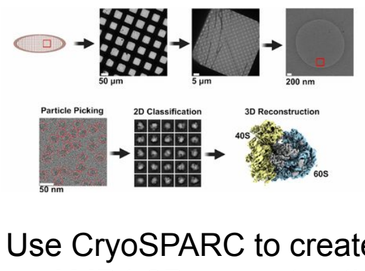
Purification + Imaging Use cryogenic electron microscopy to image purified and stalled ribosomes



Amplify and modulate AMD1 C-tail where stalling is likely with various primer locations



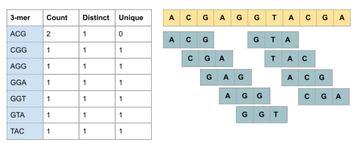
Select successful primer combination and optimal supplements



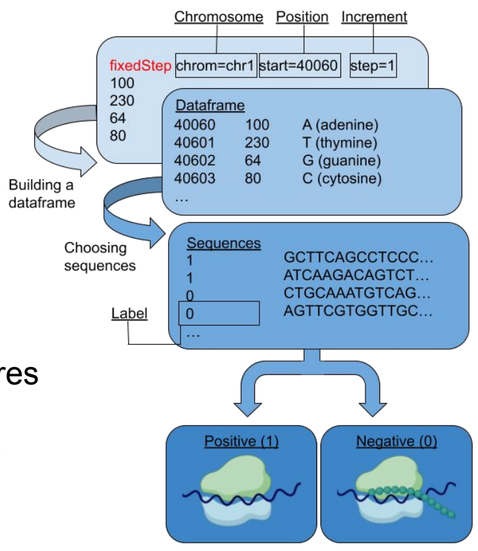
Use CryoSPARC to create an AMD1 3D reconstruction

2. Computationally identify amino acids influential to stalling

Data: Series GSE74511
- GSM1921994
- GSM1921995
- GSM1921994



3-mer	Count	Distinct	Unique
ACG	2	1	0
CGG	1	1	1
AGG	1	1	1
GGA	1	1	1
GGT	1	1	1
GTA	1	1	1
TAC	1	1	1



→ Normalize stall scores from ribosome profiling data
→ Map to human genomic data
→ Apply k-mer encoding and random forest regression
→ Extract sequences correlating with extreme stall scores

Higher ribosome coverage ∝ **Higher stalling score**

Fig 2. Methods Overview. *Image created by finalist using BioRender, 2025*

Results

Optimizing Experimental Conditions

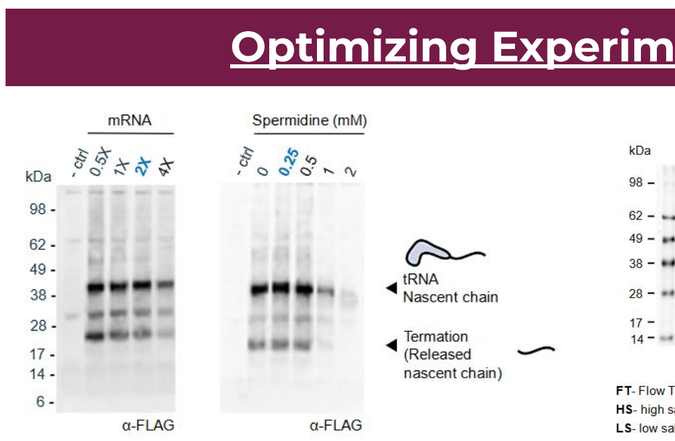


Fig 3. Optimized conditions show higher yield by reducing ribosome crowding and offering a strong band at the 38 kDa band, indicating the tRNA-AMD1 nascent chain complex. *Image taken by finalist, 2025.*

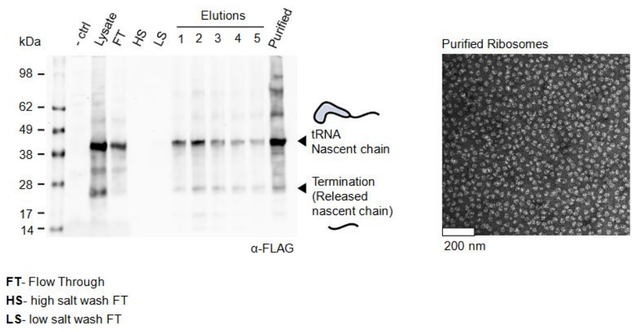
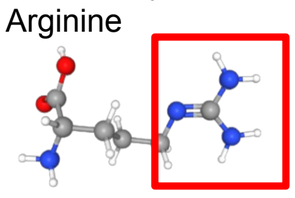


Fig 4. Successful purification of stalled ribosomes expressing the AMD1 C-tail with aligned bands and even distribution of purified ribosomes indicated by negative stain. *Image taken by finalist, 2025.*

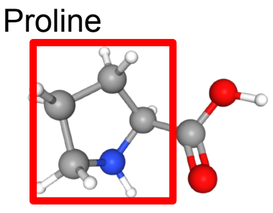
Identified Influential Amino Acids

Structurally Identified Influential Amino Acids

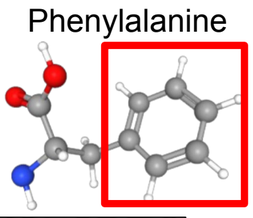
Arginine



Proline



Phenylalanine



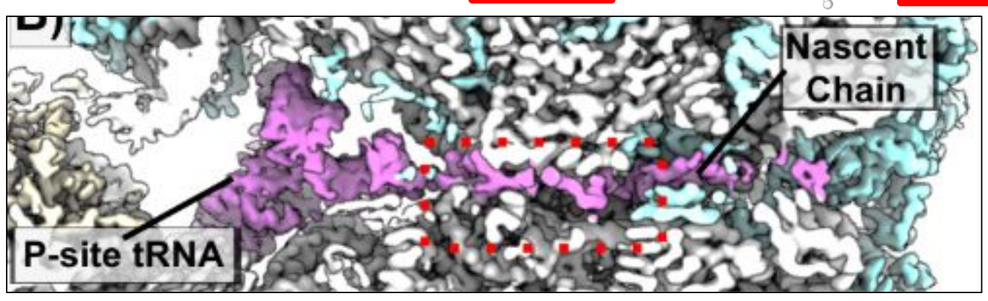


Fig 5. Nascent chain for AMD1 C-tail interacting with ribosome exit tunnel interior and P-site tRNA. *Images taken from PubChem and by finalist, 2025.*

Computational Analysis

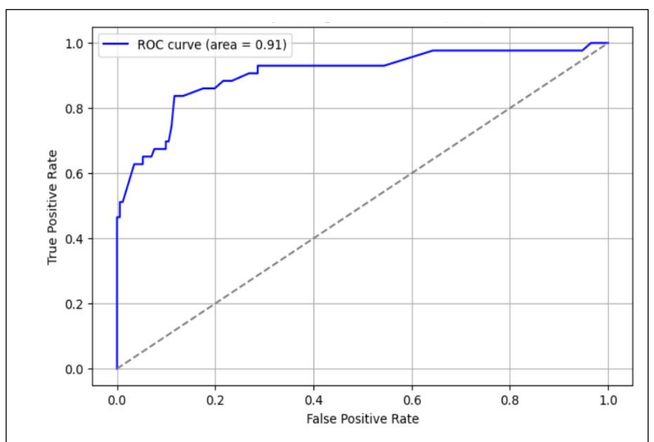


Fig 6. Area under ROC curve (AUC) shows high accuracy of 0.91 across thresholds. *Image taken by finalist, 2025.*

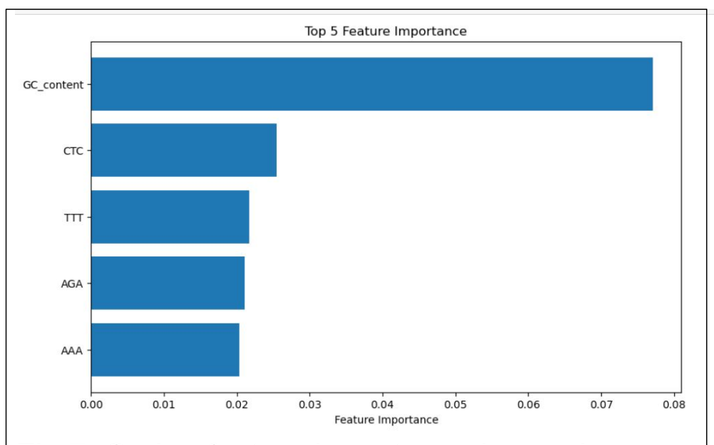
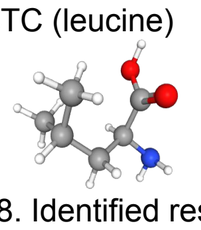


Fig 7. Codon feature importance by random forest regression. *Image taken by finalist from Jupyter Notebook, 2025.*

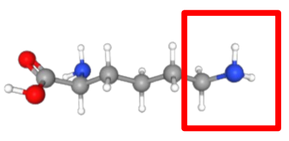
Accuracy	**91%**
F-1 Score	**90%**
Precision	**92%**
Recall	**91%**
AUC ROC	**91%**

Feature importance validates benchtop results by highlighting **charge** and **aromatic** rings, showing that **Leucine**, **Arginine**, **Lysine,** and **Phenylalanine** influence stalling in the MCF-7 genome.

CTC (leucine)



AAA (lysine)



TTT (phenylalanine)

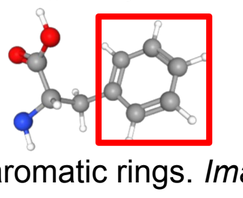


Fig 8. Identified residues tend to be charged or have aromatic rings. *Images taken from PubChem by finalist, 2025.*

Conclusions

Optimized experimental conditions using a 3' UTR reverse primer, reducing ribosome crowding, and increasing translational efficiency, reveal a **previously unknown structure**, which is validated by a **novel** random forest regression-based algorithm, **filling two critical knowledge gaps** present today.

1. **Large or hydrophobic side chains can induce stalling by interacting with the exit tunnel**
2. **Integrates WIGGLE, genomic data, and machine learning to analyze stalling trends**

These results establish a workflow to **improve accessibility to future ribosome stalling studies**, paving the way for designing **more precise inhibitors** in **cancer therapeutics**.

Future Work

Conduct **mutagenesis experiments** by mutating residues to evaluate influence of a specific residue

Leveraging the model's learning strength by expanding the training dataset to work with more profiling experiments (>3000), varying cancer lines, different 'stalling' thresholds (than >0.75 and <0.25)

Integrate entropy calculations to account for the **influence of mRNA secondary structures**

Key References

[1] Casero, R. A., Murray Stewart, T., & Pegg, A. E. (2018). Polyamine metabolism and cancer: treatments, challenges and opportunities. Nature Reviews Cancer, 18(11), 681–695. https://doi.org/10.1038/s41568-018-0050-3

[2] Zhang, S., Hu, H., Zhou, J., He, X., Jiang, T., & Zeng, J. (2017). Analysis of Ribosome Stalling and Translation Elongation Dynamics by Deep Learning. Cell Systems, 5(3), 212-220.e6. https://doi.org/10.1016/j.cels.2017.08.004

[3] Shao, B., Yan, J., Zhang, J., Liu, L., Chen, Y., & Buskirk, A. R. (2024). Riboformer: a deep learning framework for predicting context-dependent translation dynamics. Nature Communications, 15(1). https://doi.org/10.1038/s41467-024-46241-8

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