

# Nanopore direct RNA sequencing confirms coordinated base modifications in the T-loop of *S. cerevisiae* tRNA

Niki K. Thomas<sup>1</sup>, Ethan A. Shaw<sup>2</sup>, Robin L. AbuShumays<sup>3</sup>, Joshua D. Jones<sup>4</sup>, Abigail L. Vaaler<sup>2</sup>, Kristin Koutmou<sup>4</sup>, Mark Akeson<sup>3</sup>, David M. Garcia<sup>2</sup>, Miten Jain<sup>1,5</sup>

<sup>1</sup>Department of Bioengineering, Northeastern University

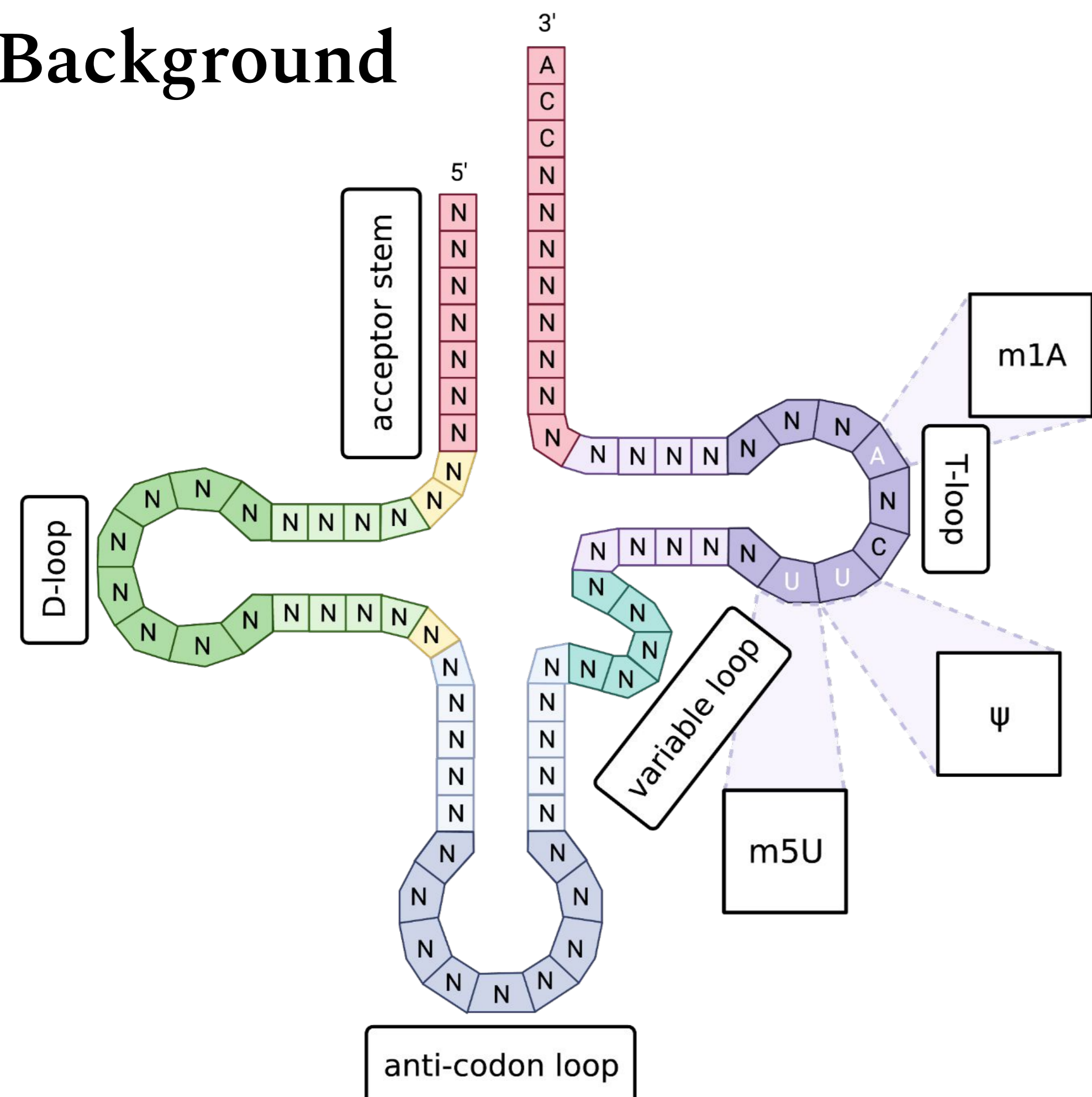
<sup>2</sup>Institute of Molecular Biology and Department of Biology, University of Oregon

<sup>3</sup>Biomolecular Engineering Department, University of California, Santa Cruz

<sup>4</sup>Department of Chemistry, University of Michigan

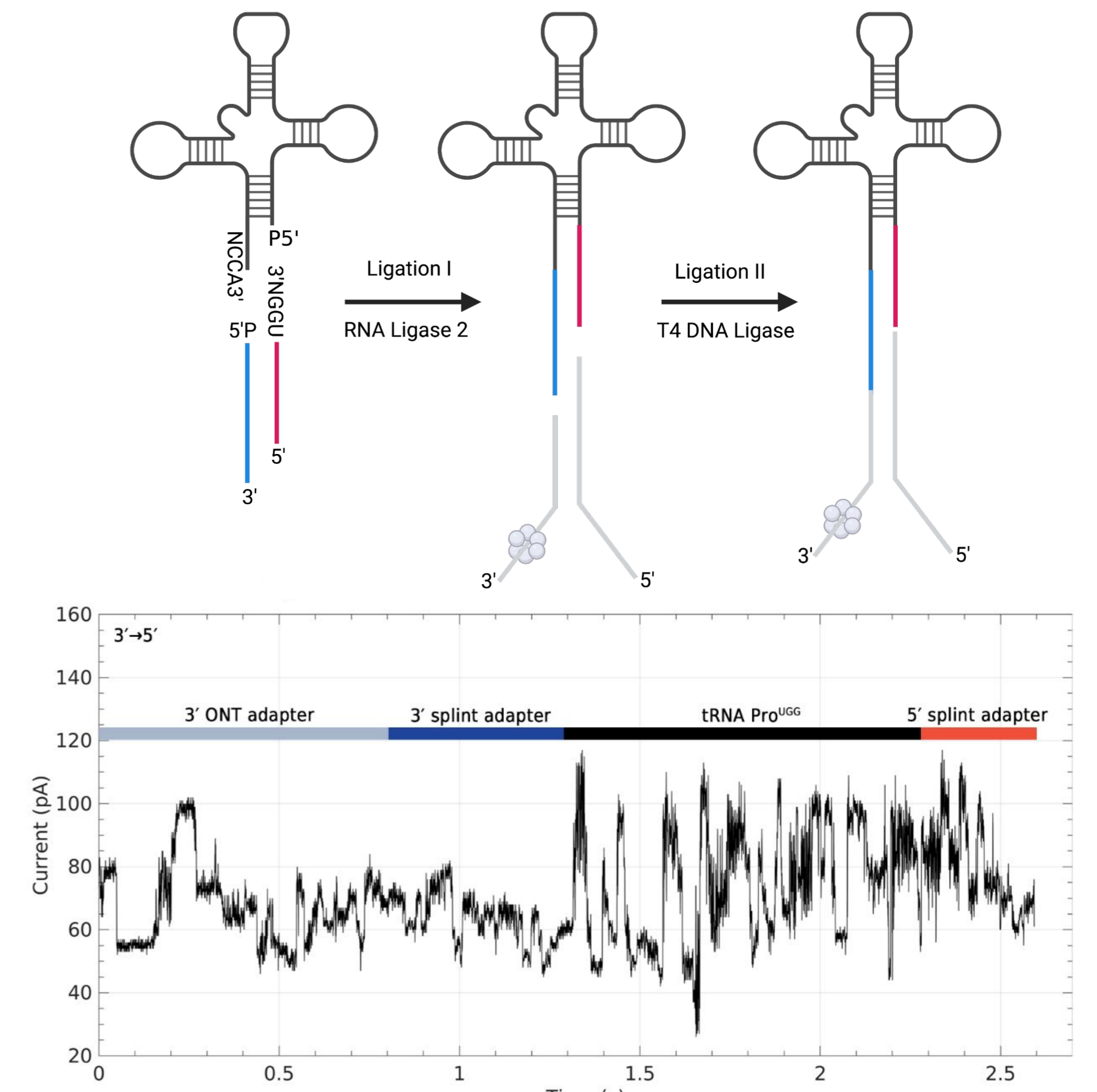
<sup>5</sup>Department of Physics, Northeastern University

## Background



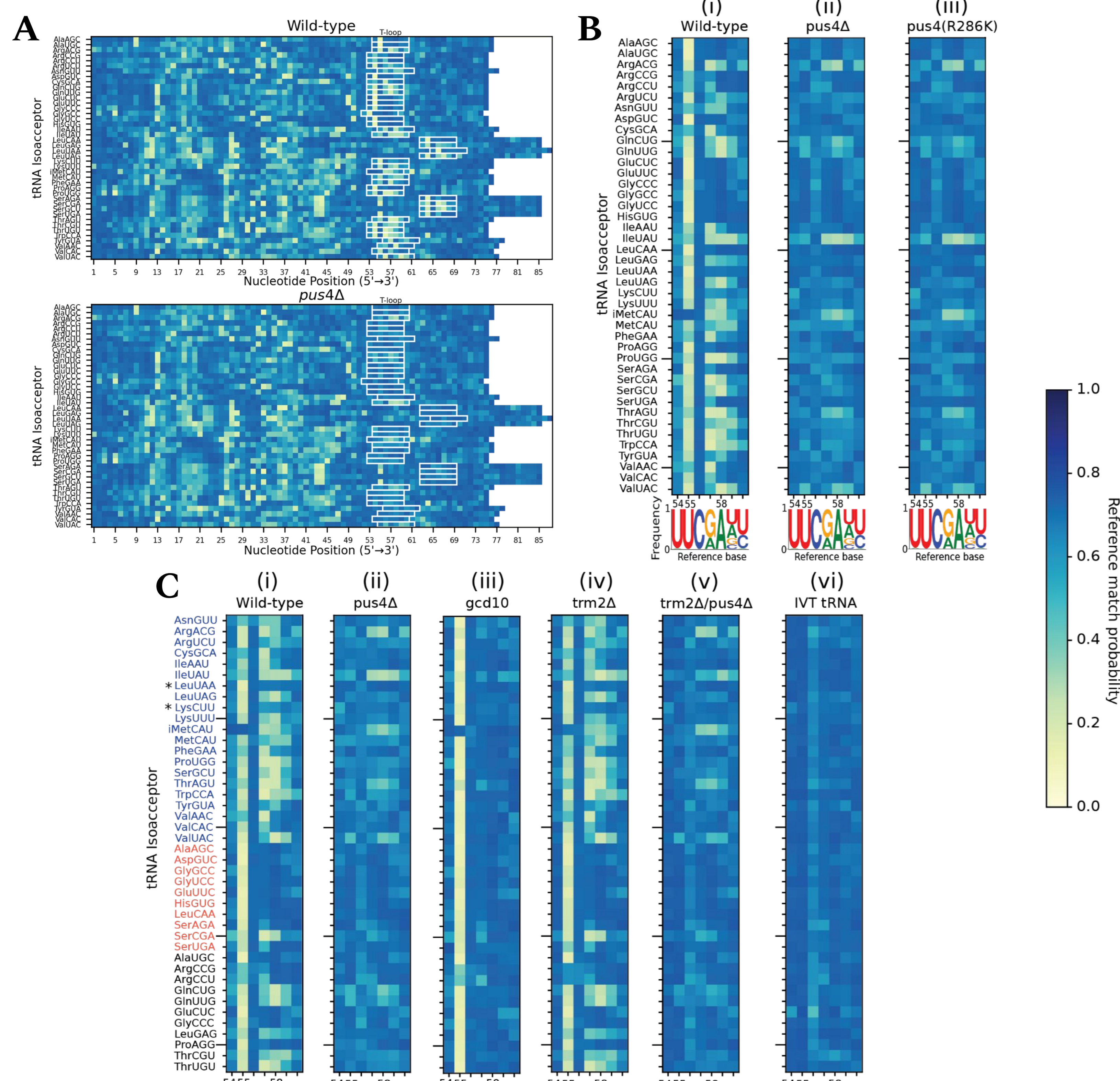
- Three nucleoside modifications in the tRNA T-loop are conserved across life: 5-methyluridine (m5U), pseudouridine (ψ), and N<sup>1</sup>-methyladenosine (m1A).
  - **T-loop modifying enzymes in *S. cerevisiae*:**
    - m5U catalyzed by **trm2**
    - ψ-55 catalyzed by **pus4**
    - m1A catalyzed by **gcd10/gcd14**
- In *S. cerevisiae* their addition is coordinated in a modification circuit, as has been demonstrated using NMR spectroscopy (Barraud 2019; Yared 2022)
- Nanopore direct RNA sequencing is capable of detecting certain modifications like ψ and m1A
- We applied Nanopore Direct RNA sequencing to investigate this modification circuit in all 42 *S. cerevisiae* tRNA isoacceptors

## Experimental Approach

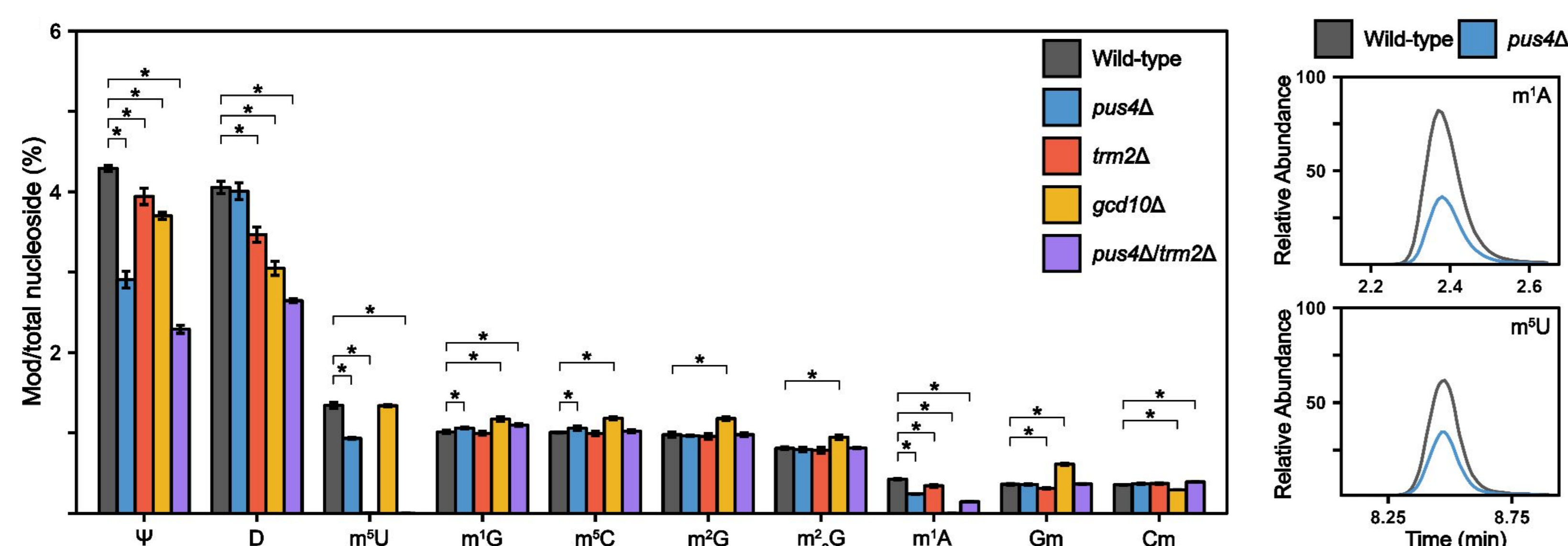


## Results

A) Base miscalls are concordant with potential modifications in all 42 *S. cerevisiae* cytosolic tRNA. B) Pseudouridine promotes the addition of m1A across most, but not all, isoacceptors. C) m5U has a modest effect on neighboring modifications.



Mass spectrometry confirms changes in the presence of m5U, ψ, and m1A between conditions, as predicted by Nanopore direct RNA sequencing.



## Conclusions

- We reinforce prior work (Smith 2015; Thomas 2021) on a method of adapting tRNA for Nanopore direct RNA sequencing.
- We validate ψ-55 and m1A-58 base miscall derived modification predictions with enzymology and mass spectrometry.
- We confirm findings that ψ-55 generally promotes m1A modification in the T-loop, suggesting order with some exceptions.
- Loss of m5U is predicted to mainly affect pseudouridylation of two tRNAs, and modestly affects m1A modification in some tRNAs. While m5U is not observable by sequencing, mass spectrometry shows that pseudouridylation promotes some m5U modification.
- We reinforce that Nanopore direct RNA sequencing can record the sequence and abundance of tRNAs (Thomas 2021).

## References

- Barraud, P., et al. (2019). <https://doi.org/10.1038/s41467-019-11356-w>  
 Yared, M., et al. (2022). <https://doi.org/10.1101/2022.12.16.520695>  
 Smith, A., et al. (2015). <https://doi.org/10.3389/fbioe.2015.00091>  
 Thomas, N., et al. (2021). <https://doi.org/10.1021/acsnano.1c06488>