Mechanistic insights into substrate recognition by Pseudouridine Synthases

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1 Introduction

Pseudouridine, also known as the 'Fifth nucleotide' is an RNA modification that isomerises uridines and is present in all RNA classes. Pseudouridylation is carried out by a family of enzymes called pseudouridine synthases (PUS) and each PUS has different substrate targets (**Fig. 1**)[1]. However, the underlying mechanism governing the selection is not clear. We aim to elucidate their structures, catalytic activities, and substrate-selectivity mechanisms, particularly for defined uridine positions within tRNAs.

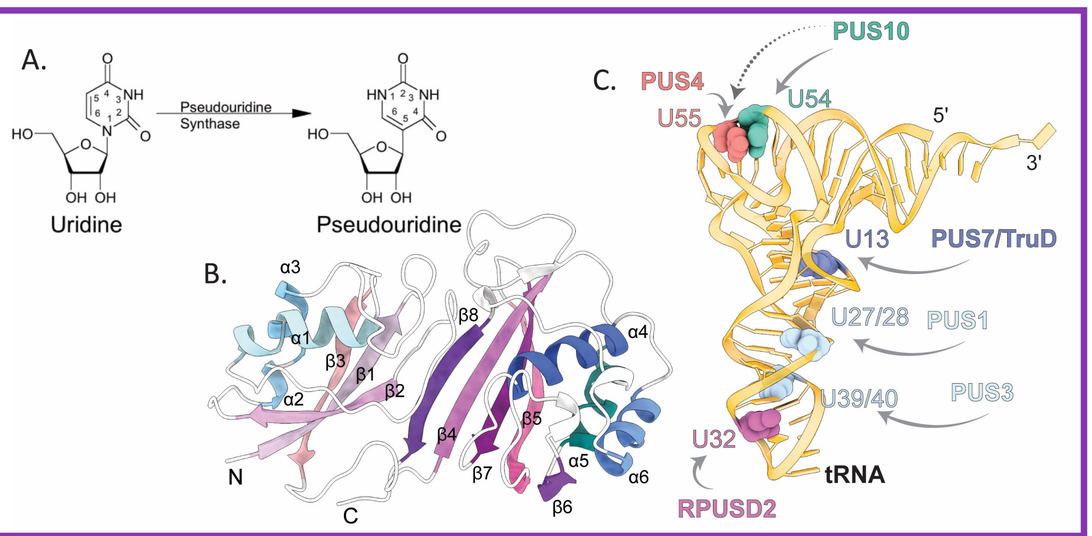

Figure 1: PUS activity and core structure. Figure 1A: Conversion of uridine to pseudouridine. Figure 1B: A cartoon representation of PUS core structure. Figure 1C: A cartoon representation of a tRNA and the conserved pseudouridine sites and the responsible PUS are indicated[1].

2 Methods and Results

Protein A Localisation

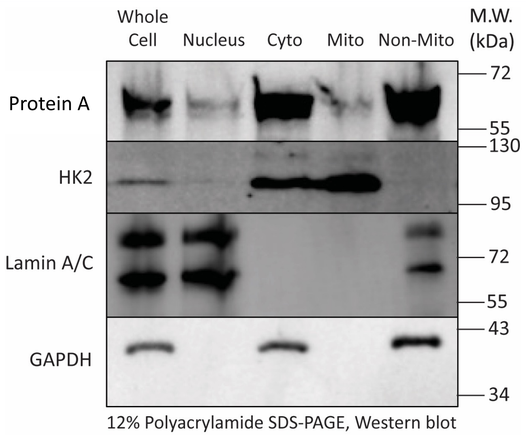

Figure 2: Cellular localisation of Protein A in the MDA-MB-231 cell line. Fractionation controls include HK2 (mitochondria), Lamin (nucleus) and GAPDH (cytosol).

tRNA Production using In vitro transcription

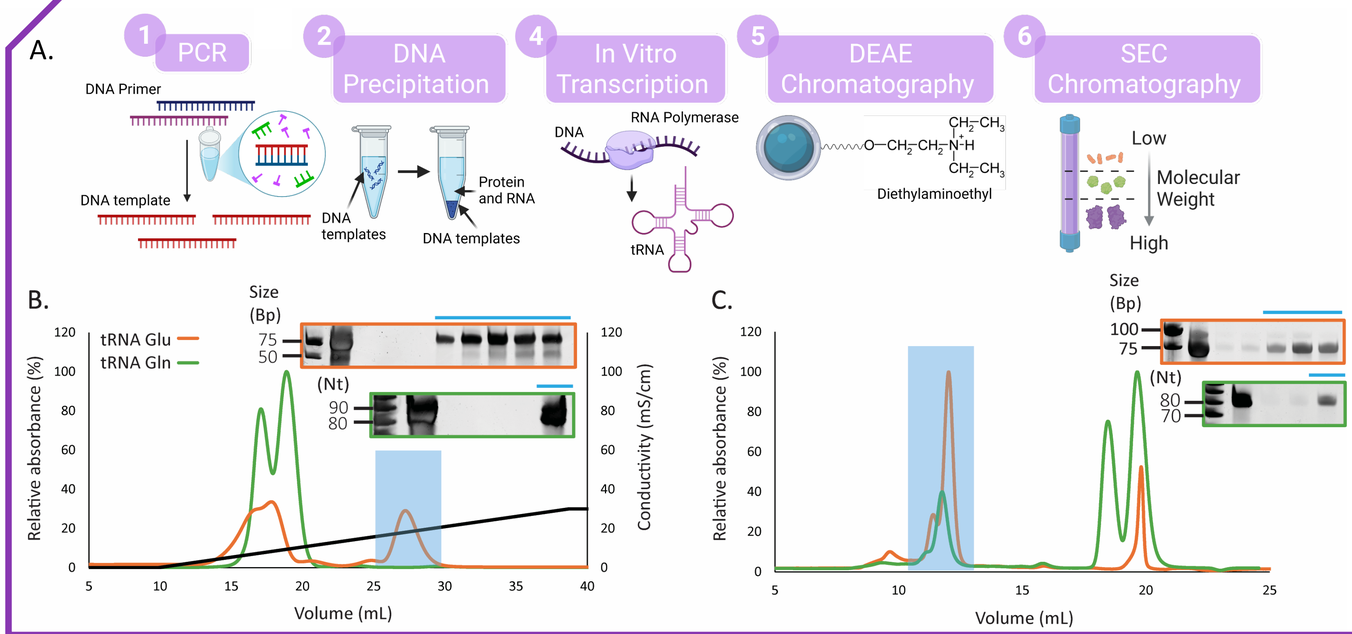

Figure 3: tRNA production and purification. **A.** A scheme of the production pipeline using Primerize to generate template and followed by IVT and purification. **B-C.** Chromatograms of weak anion exchange (**B**) and size exclusion (**C**). Purified tRNAs were visualised in gels. Fractions with tRNAs are highlighted.

PUS binds to tRNAs

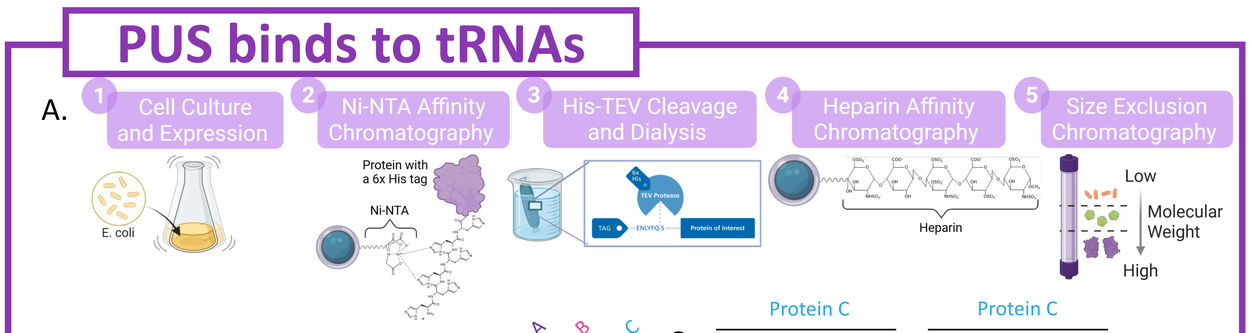

Figure 4: Biophysical characterisations of PUS interaction with tRNAs. **A**. Recombinant protein production pipeline of PUS. **B**. Purified protein quality in size exclusion chromatography (S200 pg 26/600 SEC column) and in denaturing SDS PAGE (shown in the inset). **C**. Electrophoresis mobility shift assay of PUS binding to different tRNAs. **D-E**. Affinity determination of PUS to Cy5-tRNA^{Gln} using the flow dispersion analysis (FidaBio Neo platform). **F**. Hydrodynamic radius analysis of PUS and PUS-tRNA complex (NanoTemper). Inset: Tm (°C) of PUS and PUS-tRNA complex.

Specific PUS-dependent pseudouridylation

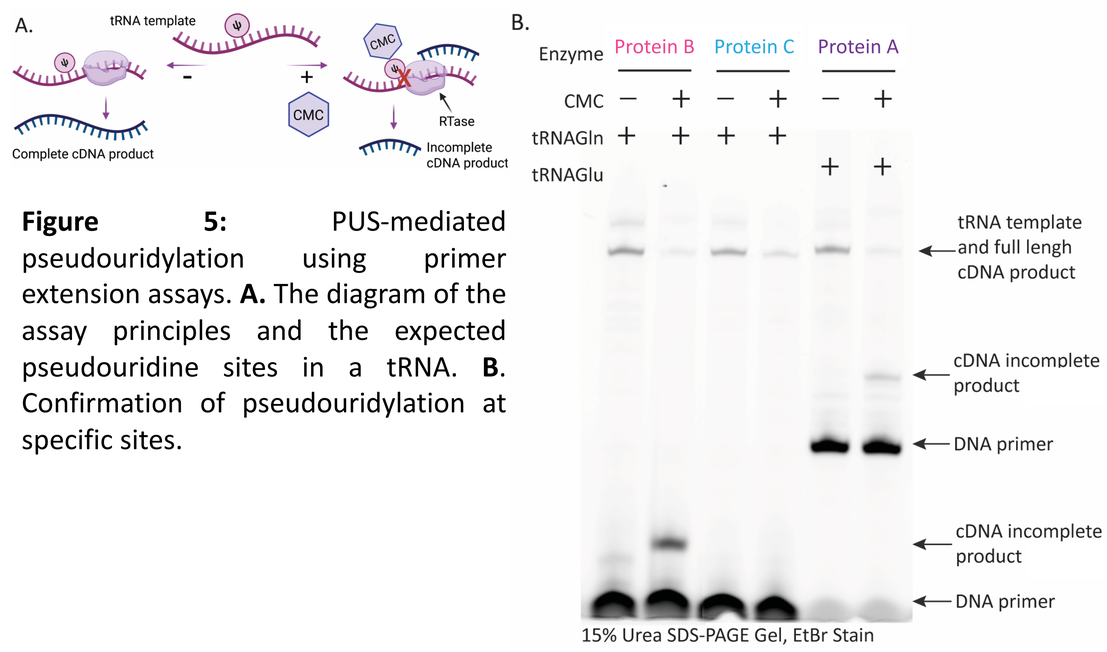

Figure 5: PUS-mediated pseudouridylation using primer extension assays. **A.** The diagram of the assay principles and the expected pseudouridine sites in a tRNA. **B.** Confirmation of pseudouridylation at specific sites.

Structural Analysis

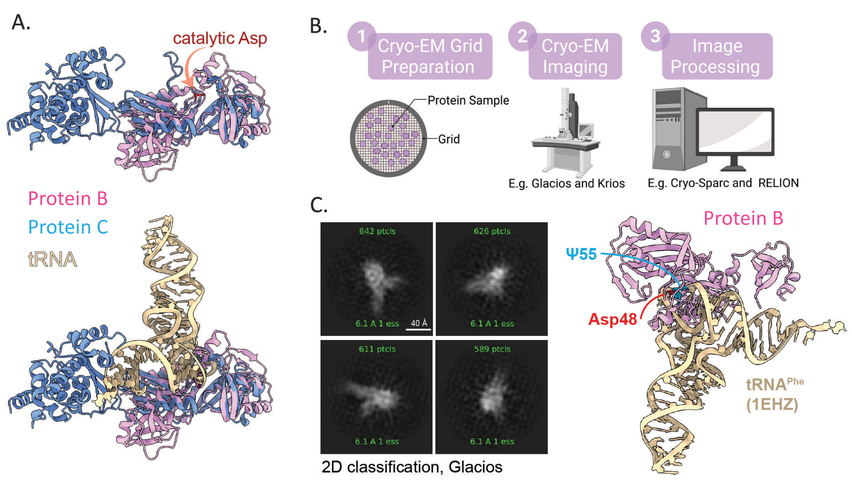

Figure 6: Structural analysis of Protein B and Protein C with tRNA. **A.** Structural impositions using deposited structures of Protein B and Protein C. **B.** Cryo-EM pipeline. **C.** 2D classes of Protein B bound to tRNA^{Glu} (data collected from a Glacios microscope at Solaris, Poland).

3 Conclusions

- A production pipeline has been established to produce PUS enzymes and tRNAs.
- In vitro analysis of the activity and binding of PUS enzymes to tRNA has been assessed.
- Cryo-EM of Protein B reveals the position-specific substrate preferences to tRNA and potentially Protein C to tRNAs.
- By understanding the molecular basis for pseudouridine synthase action; We can understand the underlying mechanism of pseudouridine sites selection at transcriptome level.

Acknowledgement

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References

1) Spenkuch, F., Motorin, Y. & Helm, M. Pseudouridine: Still mysterious, but never a fake (uridine)! RNA Biol 11, 1540–1554 (2014).