Molecular Dynamics Studies of the Ribosome CAR Surface

Carol Dalgarno[1,4], Abdelrahman Elsayed[2,5], Jack Kwon[1], Jungwoo Cho[1], Kristen Scopino[1], Daniel Krizanc[2], Kelly M. Thayer[2,3], and Michael P. Weir[1]

[1]Department of Biology, [2]Department of Mathematics and Computer Science, [3]Department of Chemistry, [4]cdalgarno@wesleyan.edu, [5]aelsayed@wesleyan.edu
Wesleyan University, Middletown CT 06459

Introduction

The molecular mechanisms by which ribosomes modulate protein translation are not well understood. Pronounced $(GCN)_n$ periodicity in yeast ORFs suggests that rRNA nucleotides near the A-site of the ribosome may base pair with mRNA nucleotides entering the decoding center. When the codon directly downstream of the A-site (the +1 codon) has the sequence GCU, the +1 codon interacts with the C1054-A1196-R146 (CAR) interaction surface of the ribosome through hydrogen bonding. This transient interaction between the ribosome and the mRNA likely plays a role in translation efficiency. To determine whether hydrogen bonding and stacking of the CAR surface is modulated by the mRNA sequence, we conducted Molecular Dynamics (MD) simulations of the decoding center with different +1 codon substitutions.

Background and Methods

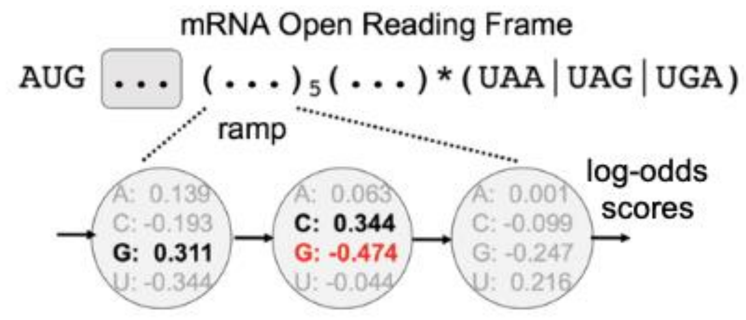

Fig 1. GCN repeats are overrepresented in the ramp region of highly expressed yeast genes.

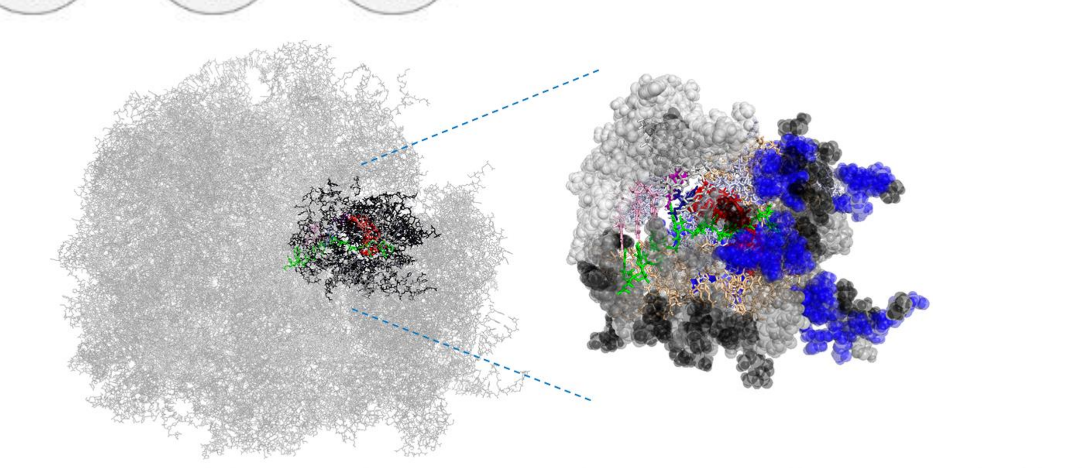

Fig. 2. 495 residue subsystem The yeast ribosome (left) is composed of a large and small subunit that associate to translate mRNA into protein. A 495 residue subsystem (right) is used in MD simulations for computational efficiency. To preserve the biological validity of this subsystem, a restraint force is placed on residues around the A-site. Cryo-EM structure PDB 5JUP [1].

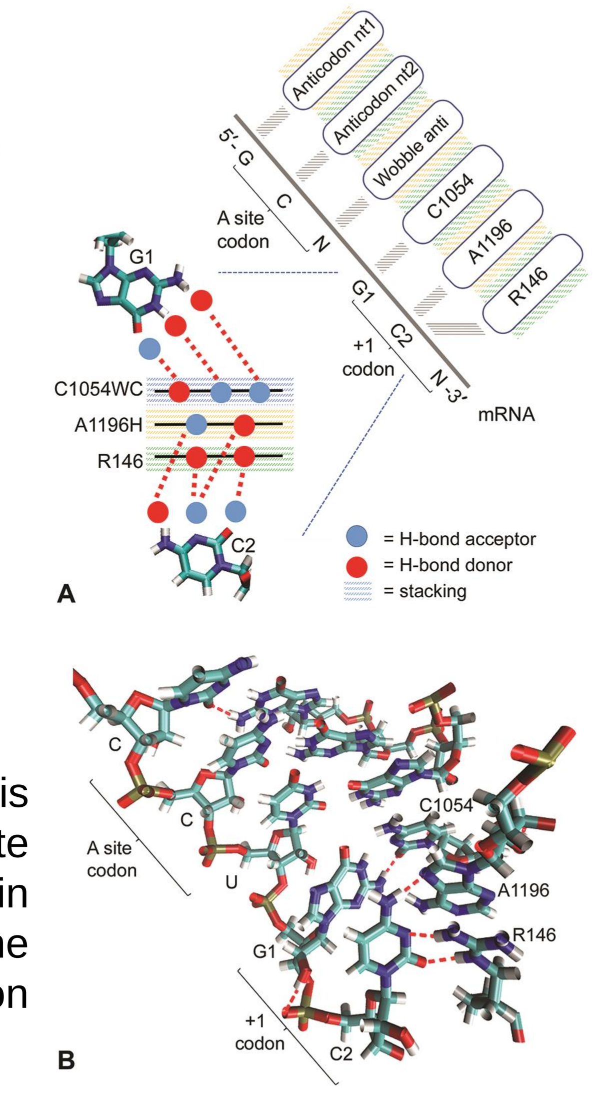

Fig. 3. GCU mediates hydrogen bonding between the mRNA and the CAR surface shown schematically (A) and in a simulation snapshot (B). G1 hydrogen bonds to C1054 through its Watson-Crick edge, and C2 hydrogen bonds to A1196 through its Hoogsteen edge and to the guanidinium group of R146. The CAR surface is anchored to the wobble tRNA, and the three CAR residues form a stable interaction surface through pi stacking of the nitrogenous bases of C1054 and A1196 and the guanidinium group of R146 [3].

Fig 4. 16 NNU +1 codon substitutions made using t-LEaP [2]

GGU	CGU	AGU	UGU
GCU	CCU	ACU	UCU
GAU	CAU	AAU	UAU
GUU	CUU	AUU	UUU

Results

Hydrogen Bonding in NNU Mutants

Fig. 5. A) A comparison of average hydrogen bonding for all 16 codon sequences with a U at position 3. **B)** Summed hydrogen bonding by mRNA residue decreases when +1 codon sequence deviates from GCU. **C)** Cross registration hydrogen bonds from position 1 of the +1 codon (N1) to tRNA nt 34, C1054, and A1196 are affected by the sequence at N1, and to a lesser extent by the sequence at N2.

RMS2D Substates

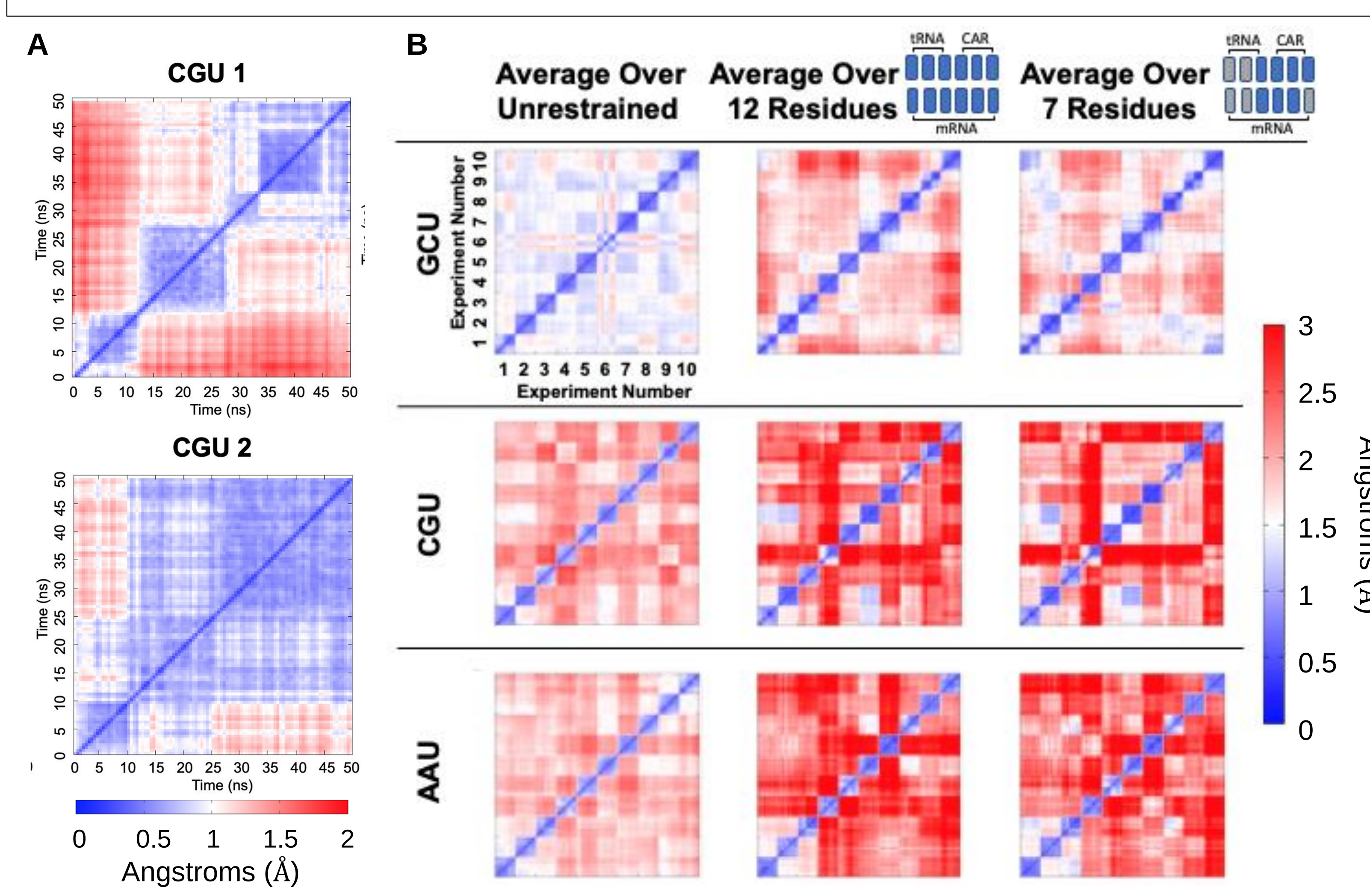

Fig 6. A framewise comparison of RMSD reveals distinct substates within experiments (A) as well as shared substates across experiments (B).

Dynamic Cross Correlation

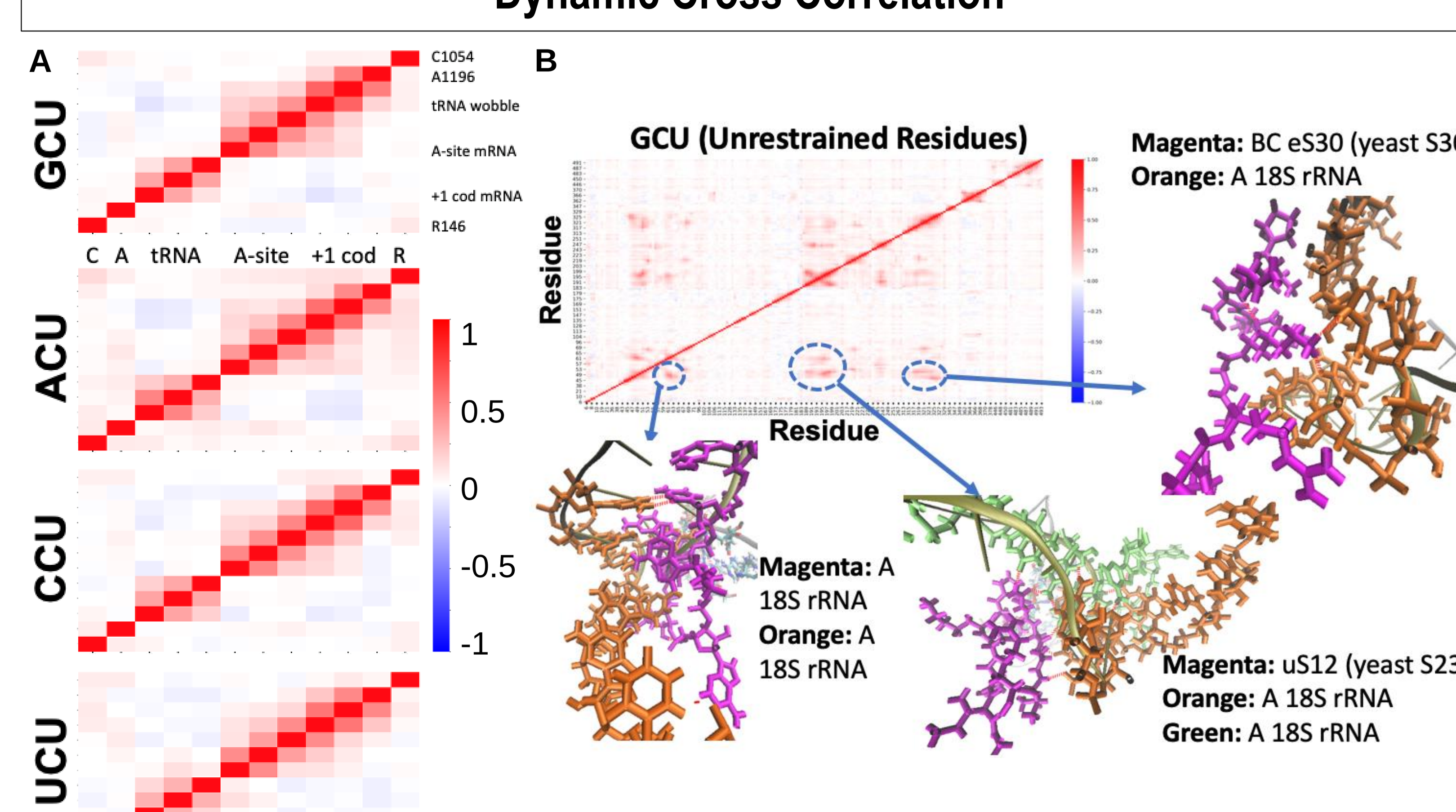

Fig 7. A) Correlated motions of residues near the A-site (x-axis and y-axis: C1054, A1196, tRNA, A-site and +1 codon mRNA, and R146). **B)** Correlated motions of rRNA chains and ribosomal proteins. Red = correlation, Blue = anti-correlation

Conclusion and Future Directions

- Disrupting mRNA $(GCN)_n$ periodicity reduces H-bond interactions with CAR, suggesting that ramp region codon sequence could tune translation efficiency.
- RMS2D shows variable substates across substitutions
- DCC reveals motional correlation of decoding center residues and of rRNA chains and ribosomal proteins.
- Perform k-means clustering of MD trajectories to identify favorable conformations of the subsystem and their correlation with mRNA sequence.
- Use MD to simulate the effect of stress modifications of nt34 and R146 on mRNA-CAR interaction.

References

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Acknowledgements

We would like to thank David Beveridge, David Langley, Ishita Mukerji, Colin Smith, Irina Russu, Abhilash Jayaraj, Elliot Williams, Will Barr, Audrey McMahon, and Clara Nachmanoff for fruitful discussions, and Henk Meij for technical assistance with Wesleyan's high-performance computing cluster. This work was supported by the McNair Program, Wesleyan Research in the Sciences, NIH grants 1R15GM096228 to MPW and R15 GM128102-01 to KMT, and NSF grants CNS-0619508 and CNS-0959856 to Wesleyan University.