Investigating Covalent Small Molecule Interactions with Human RNA

Annika Bridge, Alexandra Khitun, and Kevin M. Weeks[1]

[1]Department of Chemistry, UNC Chapel Hill

Significance

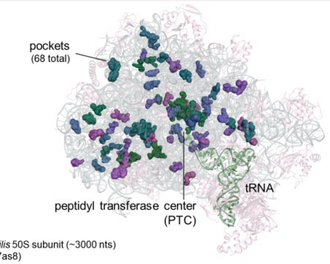
Most current drugs and therapeutics covalently engage with and target proteins. As described by the Central Dogma of Biology, DNA (deoxyribonucleic acid) in the nucleus of a cell is transcribed into RNA (ribonucleic acid) which then serves as a messenger molecule for protein synthesis. However, while ~70% of the mammalian genome is transcribed into RNA, less than 2% is protein-coding. This non-coding RNA contributes to additional cellular functions. Thus, RNA is a desirable target for therapeutics as it is transcribed from a larger part of the genome than what is translated into proteins and may serve as a regulator for cellular mechanisms or be related to particular disease states.

Vision

Using an RNA probing technology invented in the Weeks laboratory and chloroacetamide compounds, we will locate binding locations in RNA that could be used as targets for future therapeutics.

RNA as a Drug Target

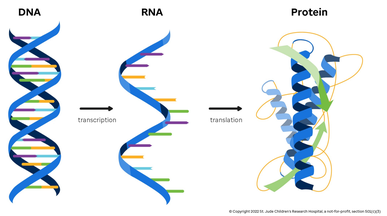
RNA tertiary structure forms pockets that can bind small molecules



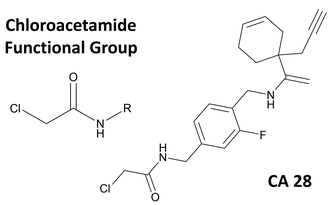
B. subtilis 50S subunit (~3000 nts)
(PDB: 7as8)

Chloroacetamides selectively bind RNA

The Central Dogma of Biology[3]



Chloroacetamide Functional Group

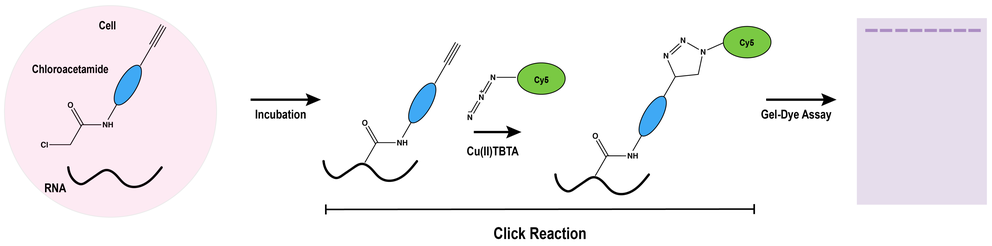


CA 28

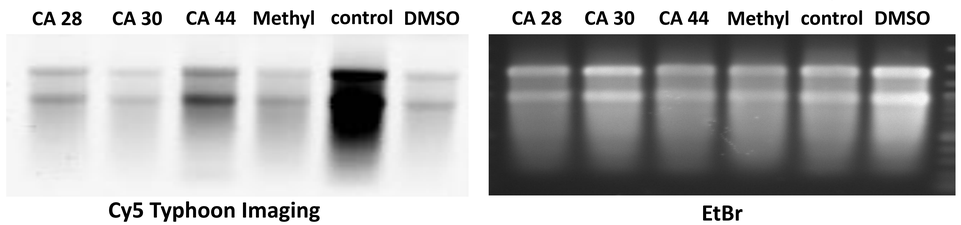
Methods and Results

Gel-Dye Assays screen for RNA Binding

RNA is probed and labeled through a click reaction



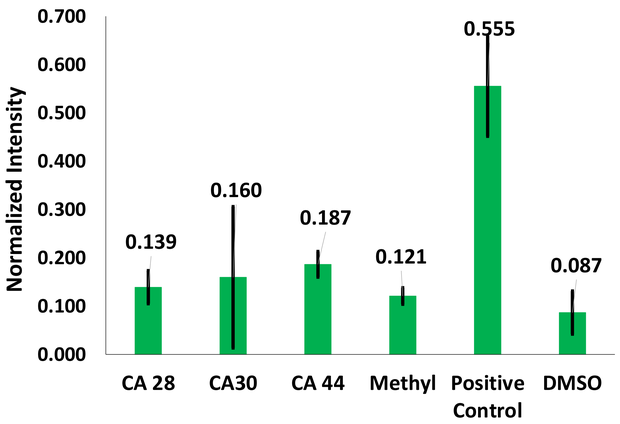
Gels imaged with Cy5 fluorescent dye and EtBr



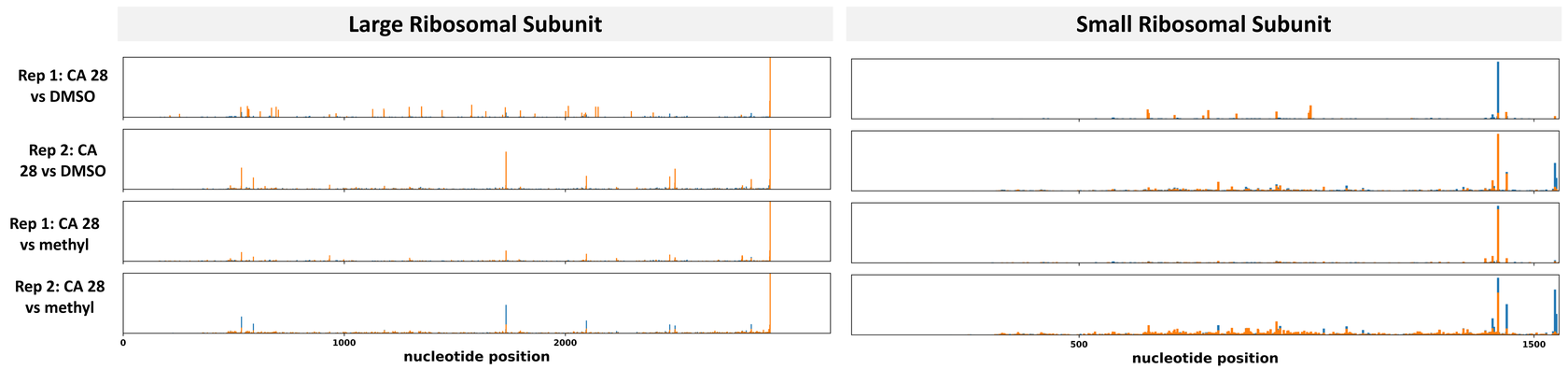
Cy5 Typhoon Imaging

EtBr

Average Normalized Fluorescence



Binding Sites are identified through DNA Sequencing



Conclusions

- Binding was observed for CA 28, CA 30, CA 44, with CA 44 displaying the greatest binding ability in gel-dye assays.
- Early replicates do not indicate specific binding sites for CA 28.
- Chloroacetamide compounds bind RNA selectively with limited affinity.

Future Research

- Determine RNA binding for the compound iodoacetamide which interacts non-selectively with RNA.
- Investigate binding affinity of iodoacetamide through binding competition assays.
- Elucidate structure of identified binding sites through structural probing experiments.

REFERENCES

[1] Boike, L., Henning, N.J. & Nomura, D.K. Advances in covalent drug discovery. *Nat Rev Drug Discov* **21**, 881–898 (2022). https://doi.org/10.1038/s41573-022-00542-z

[2] Zou, Z.,Wei, J., He, C. (2023). New horizons of regulatory RNA.*Fundamental Research* ,3, 760-762, 26673258. https://doi.org/10.1016/j.fmre.2022.12.001.

[3] St. Judes Children's Research Hospital. (2022). *The Central Dogma of Biology*. The Central Dogma. Retrieved from https://learngenomics.dev/docs/biological-foundations/the-central-dogma/.