Structural Characterization of a Small Molecule-RNA Triple Helix Complex

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Three-dimensional (3D) structures of drug targets have been essential in drug design and discovery. Cryogenic-electron microscopy (cryo-EM) is a revolutionary method that has been successful for elucidating the 3D structures of macromolecules, including small RNAs.

However, only a handful of RNA-only 3D structures have been solved and none are in complex with a drug-like small molecule. Human metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is a nuclear-retained long non-coding RNA (lncRNA) with a 3′-terminal triple helix. The triple helix contributes to the overall stability of MALAT1 by preventing 3′ degradation, resulting in the nuclear accumulation of MALAT1. This over accumulation contributes to the onset and progression of disease, making the triple helix an alluring drug target. Small molecule therapeutics are rapidly expanding due to their deliverability, uptake, and tunability. Promising small molecule libraries comprised of the diphenylfuran (DPF) and diminazene (DMZ) scaffolds, which are known triplex-binding molecules, were developed and initially characterized for their binding effects on the MALAT1 triple helix by the Hargrove laboratory. Currently, there is no 3D structure of the MALAT1 triple helix in complex with a small molecule. Herein, I am working towards solving a 3D structure of the MALAT1 triple helix in complex with DPF/DMZ small molecules. Thus far, I have grafted the MALAT1 triple helix onto previously solved cryo-EM RNA structures to improve single particle contrast and particle picking. The apo MALAT1 triple helix has been solved at a 5.2 Å resolution; this apo structure will be a reference for density and conformational changes that occur in the presence of a small molecule. I am currently working toward a higher-resolution structure while also optimizing grid conditions for the RNA-small molecule complex. Overall, this study will provide a platform for researchers to better understand how small molecules interact with the MALAT1 triple helix, providing a means to rationally design better binders.