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Atomic Force Microscopy as an Integrative Tool for Rna Structure Characterization: A Lncrna Case Study

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The secondary and tertiary structures adopted by single-stranded RNA molecules play a crucial role in determining their function in cellular processes. However, the majority of RNAs remain structurally uncharacterized. In this study, we highlight the use of Atomic Force Microscopy (AFM) to investigate RNA secondary structure, as it enables direct visualization of structural heterogeneity at the single-molecule level. Here, we developed a method for synthetizing RNA molecules with poly-A tails to tag the 3' and 5' ends. This allowed us to localize intact full-length molecules and to recognize reproducible features. Moreover, we have established an image analysis framework to extract structural information from AFM images. As a case study, we applied this methodology to the long non-coding RNA CONCR, which is implicated in sister chromatid cohesion. Our analysis reveals the structural landscape of CONCR, identifying distinct structured domains along the molecule. This work highlights the AFM advantages in deciphering the global conformation of RNAs, which in combination with other high-resolution techniques like cryo-EM and Chemical Probing can offer a deeper understanding of the functional roles of RNAs.