Exploring Structural Dynamics of In Vitro Transcribed Therapeutic mRNAs with Atomic Force Microscopy

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Background: Following recent success of the COVID-19 vaccine, mRNA technology has emerged as a versatile therapeutic platform with applications spanning infectious diseases, cancer treatments and protein replacement therapies for genetic disorders. While effective mRNA therapeutics can be designed; their structural conformation cannot yet be fully controlled, limiting the production of highly effective mRNA-based drugs. Long in vitro transcribed mRNAs tend to fold into heterogeneous ensembles of secondary structures. Short complementary regions form helical stems separated by non-base-paired loops and bulges which makes further interactions to form higher-order tertiary structures. Additionally, due to codon degeneracy, numerous synonymous mRNAs encode the same protein with each sequence adopting different structural conformation. The biological and physicochemical properties of synthetic mRNAs depend critically on their overall spatial conformation which directly dictates the lifetime of the transcript and expression levels of the designed therapeutics. Conformational heterogeneity and intrinsic dynamic of mRNA challenges both folding algorithm models and high-resolution structural techniques which often only captures a subset of its structural ensemble. Method: Here we report a method for RNA secondary structure elucidation using atomic force microscopy (AFM) together with a purpose-built AFM-image analysis pipeline to investigate the structural landscape of individual molecules of various nano-Luciferase mRNAs from our in-house collection. Results: Due to the high signal-to-noise ratio of AFM, this approach allows quantitative characterization of morphological descriptors of transcripts as structural fingerprints to classify and compare RNA secondary structures. The proposed framework detects conformational changes induced by external conditions like pH and ionic strength, and by internal chemical modifications such as N1 -methylpseudouridine. Our approach captures different features of structural ensembles. Conclusion: Better understanding of the intricate relationship between codon optimisation, thermodynamic stability and structural diversity of synthetic mRNAs would allow the design of effective mRNA-based drugs with increased stability and enhanced translational efficiency.