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ParalinearDesign: Expanding the mRNA Design Space through Near-Pareto-Optimal Sampling with Tunable Structural Constraints

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Effective mRNA vaccines require careful optimization of their RNA sequences. Highly structured RNAs exhibit enhanced stability in storage solutions and better evasion of innate immune responses during and after endocytosis. Conversely, the use of rare codons can induce ribosome stalling, triggering codon-optimality-mediated degradation and no-go decay of mRNA molecules. While a single protein sequence can be encoded by over 10^{600} codon combinations, each variant yields distinct levels of codon optimality and RNA secondary structure. Current state-of-the-art design tools, including LinearDesign and DERNA, address this by optimizing the balance between minimum free energy (MFE), which quantifies secondary structure strength, and the codon adaptation index (CAI), which favors frequent codons. However, RNA is a complex molecule regulated by over a thousand RNA-binding proteins in cells, making mRNA activity and efficacy difficult to predict based solely on these two metrics. Traditional drug development addresses such uncertainties through the screening of multiple candidates, yet existing mRNA design programs rely on deterministic optimization algorithms that produce only a single candidate per antigen sequence. To address these limitations, we developed ParalinearDesign, an extension of LinearDesign. Our algorithmic modifications enable random sampling near the Pareto-optimal front between MFE and CAI, generating codon selections that span a diverse topological space of RNA secondary structures through iterative sampling with adaptively tuned parameters. Additionally, ParalinearDesign incorporates tunable constraints for co-folding between the coding sequence and 3′ untranslated region (UTR), which promotes additional base-pairing within the RNA molecule while enhancing robust and stable folding of protective cis-regulatory elements in the 3′ UTR. This renders the structure more predictable and less susceptible to RNA regulatory factors. By facilitating the generation of diverse mRNA libraries, ParalinearDesign aligns mRNA vaccine development with industry-standard screening processes, expanding the experimental search space to enable identification of more optimal candidates and support robust, fail-safe drug design.