Despite the notable advantages that have been achieved, several challenges and limitations still persist in this area of research. First and foremost, one major limitation is that the expression of mRNA is influenced by multiple factors, including secondary structure, codon usage bias, untranslated regions (UTRs), and cellular context (Mo et al., 2025). This implies that optimizing only a single component, such as the coding sequence, is insufficient for maximizing translation efficiency. Additionally, current research is primarily focused on optimizing a single coding sequence (CDS), without considering other essential regions of mRNA that contribute significantly to overall function and stability (Xu et al., 2025). Ignoring regulatory elements and rare or context-dependent codons may lead to reduced mRNA stability and inefficient translation.

Moreover, another critical limitation is the lack of incorporation of chemically modified nucleotides in current circular RNA (circRNA) design strategies (Xu et al., 2025). Modified nucleotides such as pseudouridine or 5-methylcytidine have been shown to enhance mRNA stability, translation, and immune evasion. Their exclusion could significantly restrict the full therapeutic and functional potential of circRNA platforms. If future designs can integrate modified nucleotides based on empirical evidence, the applications of circRNA in therapeutic protein expression and gene regulation could be significantly broadened.

Given the high cost and complexity of a full redesign, I believe a more practical approach is targeted validation through a nested design strategy. This would allow for systematic testing of small modular changes while controlling costs and maintaining conceptual feasibility. Further in-depth studies into the nature and impact of modified nucleotides are essential for improving design precision and translational potential.