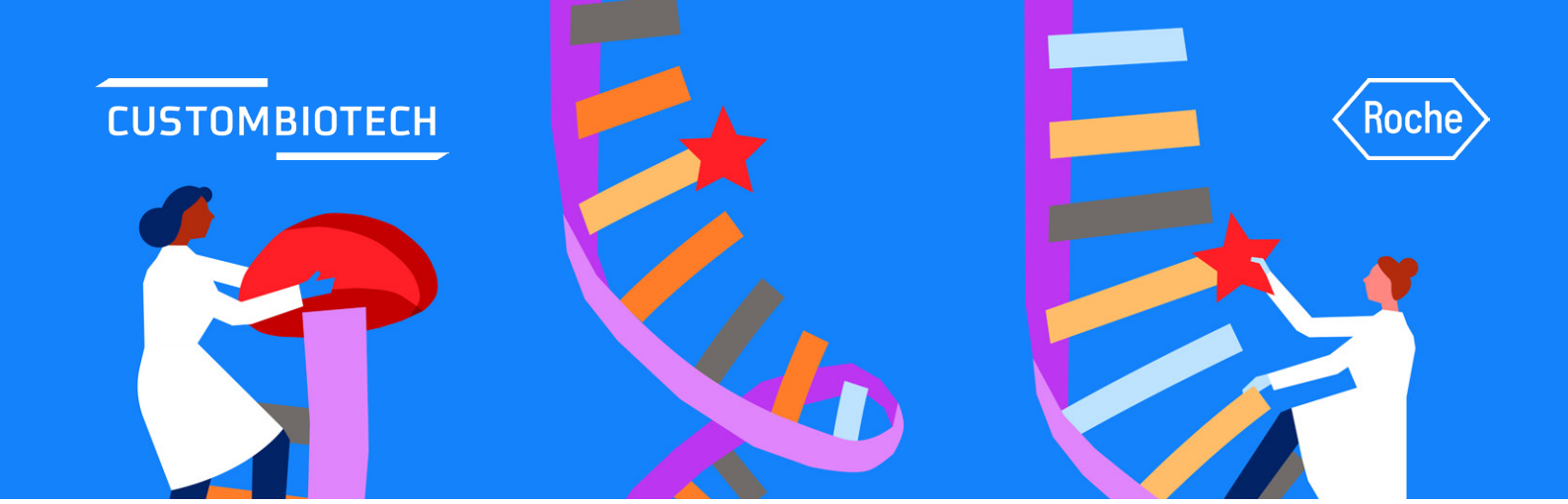
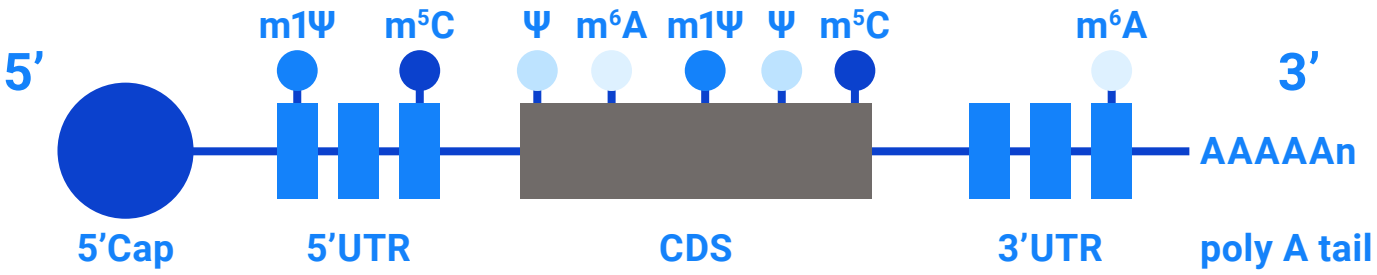


Modulating the effectiveness of mRNA therapies using modified ribonucleotides

Modified ribonucleotides can boost the safety of mRNA vaccines and therapeutics

In vitro transcribed mRNA has become an effective and scalable therapeutic modality for a wide range of disease indications after the successful development of two COVID-19 vaccines.[1] Vaccines and therapeutics based on mRNA technology work by instructing the body to make the protein of interest (e.g., a viral subunit in the case of a vaccine). However, the product works effectively only if the injected mRNA does not generate immunotoxicity.

This is where modified ribonucleotides can boost the safety of mRNA vaccines and therapeutics. The first generation of approved COVID-19 vaccines developed by Pfizer-BioNTech and Moderna Therapeutics (comirnaty® and spikevax®, respectively) are both based on mRNA containing the modified nucleotide N1-methyl-pseudouridine.[2]



Immunogenicity of mRNA

As many viruses contain nucleic acids, cells have a defense system of pattern recognition receptors (PRRs) that sense foreign nucleic acids and trigger an innate immune response by the induction of cytokines. These PRRs include the endosomal receptors TLR3, TLR7, and TLR8, and the cytosolic receptors RIG-I and MDA-5. The first three recognize double and single-stranded RNA, while the latter two recognize 5′-triphosphate-modified RNA in addition to double-stranded RNA.[3]

In vitro transcribed and exogenously delivered mRNA is intrinsically immunogenic and can cause reduced translation.[4, 5] Induction of an immune response can, in principle, be a positive stimulus for a vaccine. However, an uncontrolled immune activation can compromise safe application. Additionally, immune signalling can prevent sufficient protein translation, thereby reducing the efficacy of a vaccine or therapeutic.[5] Modifying mRNA structural elements, coding region, and polyadenylation can reduce immunogenicity as well as increase stability and translation, to a certain degree.[6, 7]

Modified Ribonucleotides

Excessive mRNA immunogenicity can be prevented by incorporating modified ribonucleotides.[1, 2] Chemical modifications of mRNA are fairly common in nature and they influence post-transcriptional gene regulation in eukaryotes. More than 140 RNA modifications have been discovered across different organisms. Of those, pseudouridine (Ψ), N6-methyladenosine (m6A), 5-methylcytidine (m5C), and 2′-O-methylation (2′OMe)are found in mammalian mRNA.[8]

Use of modified ribonucleotides in mRNA production contributes to immunogenicity reduction, as well as increased protein production.[5, 9, 10] Although the exact mechanisms by which modified ribonucleotides influence mRNA immunogenicity or protein production have not been defined, they exert their influence by altering one or more of the following properties of mRNA:

Reduced mRNA immunogenicity

- Altering RNA recognition by intracellular receptors of the immune defense system [5]
- Altering mRNA secondary structure and thereby influencing RNA-receptor interactions [11, 12]
- Altering antisense transcript synthesis by decreasing the “self-priming” effect of RNA polymerase, which reduces dsRNA [13]

Increased protein production

- By regulating functional mRNA half-life [14]
- Via increased translation [15, 16]

Choose fit-for-purpose modified ribonucleotides to enhance your mRNA technology platform

Use modified ribonucleotides from Roche CustomBiotech in the synthesis of mRNA for therapeutics or vaccines. As high-performance reagents for the manufacture of therapeutics, our modified ribonucleotides are designed to meet quality and manufacturing specifications of the biopharmaceutical industry (fit-for-purpose). They are:

- animal-origin-free (AOF)
- free of ß-Lactam-antibiotics
- extensively tested for impurities
- validated via stringent QC release and cleaning methods
- supplied with supplementary documents for regulatory submissions

Discover CustomBiotech modified ribonucleotides and talk to our team about your needs in mRNA raw materials for your manufacturing process.

Production of modified mRNA via *in vitro* transcription

Production of mRNA vaccines and therapeutics is based on the in vitro transcription (IVT) reaction. The RNA polymerases used for the IVT reaction can also incorporate modified ribonucleotides that do not significantly alter base pairing.[10] When a modified ribonucleotide is used in an IVT reaction, the regular nucleotide is replaced completely in the reaction mixture. Given that this results in a 100% substitution, use of a modified ribonucleotide must be compatible with all functional elements found in an mRNA transcript.[17]

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