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GENOMICALLY RECODED ORGANISMS (GROS): REWRITING LIFE'S CODE FOR NOVEL APPLICATIONS IN PROTEIN-BASED BIOMATERIALS ENGINEERING

J. Rithika [1], S. Rajesh [1*], S.V. Ramesh[2], S. Navinraj[1], P. Meenakshisundaram[1], R. Raghu[1], M. Kavitha[3], N. Sritharan[4]

[1]Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore 641 003, India.
[2]Division of Physiology, Biochemistry and Post-Harvest Technology, Indian Council of Agricultural Research (ICAR), Central Plantation Crops Research Institute (CPCRI), Kasaragod, Kerala, India
[3]Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore 641 003, India
[4] Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore 641 003, India.
*Corresponding author ORCID-ID 0000-0002-7000-8791

ABSTRACT

Genomically recoded organisms (GROs) represent a major advancement in synthetic biology, enabling the deliberate redesign of the genetic code to expand the functional capabilities of living systems. By combining genome-wide codon reassignment with orthogonal translation systems, GROs facilitates the incorporation of noncanonical amino acids (ncAAs) into proteins, thereby extending the chemical diversity beyond that provided by the twenty canonical amino acids. These technologies allow precise modification of translational machinery, enabling the synthesis of proteins with novel structural, catalytic, and physicochemical properties. Recent advances in genome engineering platforms, including multiplex automated genome engineering, conjugative assembly genome engineering, and CRISPR-based technologies, have accelerated the development of GROs with enhanced translational flexibility, viral resistance, and intrinsic biocontainment features. The ability to incorporate ncAAs has expanded applications in protein engineering through site-specific functionalization, multisite amino acid incorporation, and the development of proteins with improved stability and catalytic performance. Furthermore, GRO-derived proteins have emerged as promising building blocks for advanced biomaterials, including self-assembling protein polymers, functional hydrogels, bioorthogonally cross-linked materials, and stimuli-responsive systems. Despite these advances, challenges related to cellular fitness, translational efficiency, metabolic burden, evolutionary stability, and industrial scalability remain significant barriers to broader implementation. This review summarizes recent progress in GRO engineering, orthogonal translation technologies, and genetic code expansion, with particular emphasis on their applications in advanced protein engineering and biomaterial design. Additionally, the biosafety implications, current limitations, and future prospects of GRO-based technologies are discussed.

KEYWORDS: Genomically recoded organisms; Genetic code expansion; Noncanonical amino acids; Orthogonal translation systems; Synthetic biology; Protein engineering; Biomaterials.

INTRODUCTION

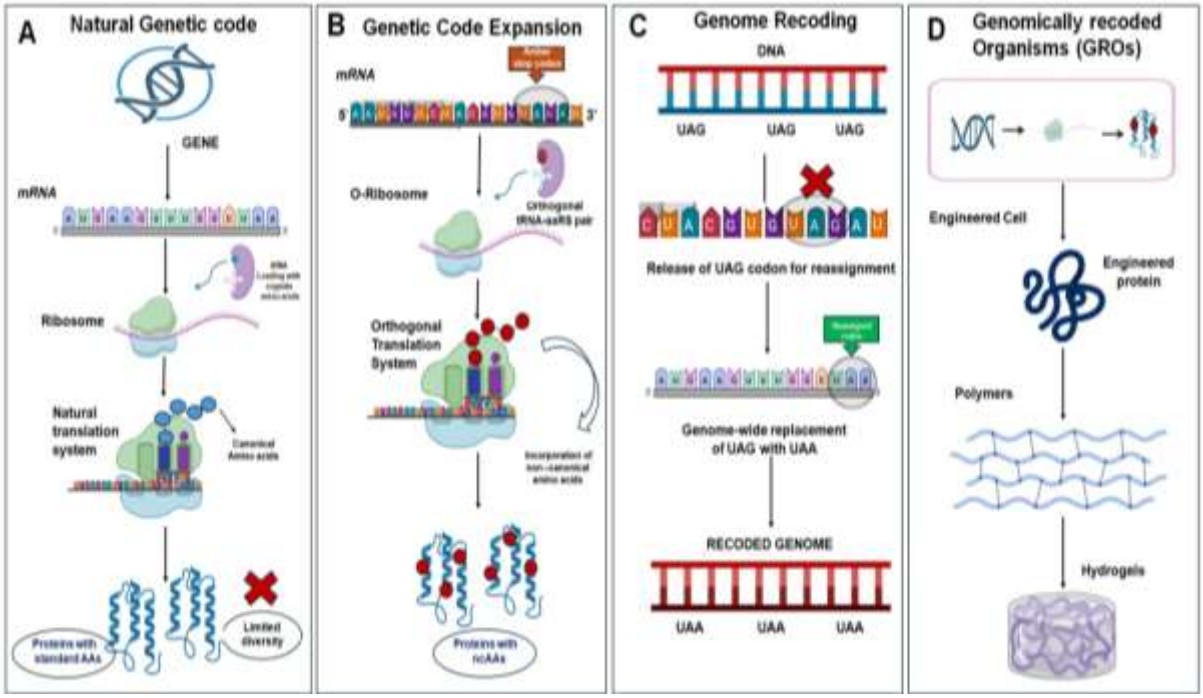
The genetic code is the blueprint that dictates how the information in nucleic acids is translated into functional proteins. In most organisms, 64 codons correspond to 20 standard amino acids and three stop signals. While this system is remarkably efficient and highly conserved, it limits proteins to the chemical functionalities provided by the side chains of standard amino acids (Chin, 2014). Many potentially useful functional groups that could expand protein diversity or catalytic abilities are simply absent in naturally occurring proteins. To address this limitation, synthetic biology has increasingly explored ways to expand or reprogram the genetic code, allowing the incorporation of noncanonical amino acids (ncAAs) into proteins (Chin, 2017) .These approaches open up exciting possibilities for introducing new chemical functionalities and broadening the scope of protein engineering (De La Torre & Chin, 2021).

Technologies for genetic code expansion (GCE) have become powerful tools for increasing the chemical diversity of proteins. These strategies often use orthogonal translation systems, which consist of engineered transfer RNAs (tRNAs) and aminoacyl-tRNA synthetases (aaRSs) that operate independently of the cell's natural translational machinery (Mukai et al., 2017).By specifically recognizing ncAAs and incorporating them at defined codons, these systems enable site-specific modification of proteins as they are synthesized. Many studies have shown successful incorporation of synthetic amino acids bearing bio-orthogonal reactive groups, fluorescent labels, or photo-crosslinking moieties (Aleksashin et al., 2020).These ncAAs provide unique chemical handles for protein labeling, structural analysis, and functional studies(J. Chen & Tsai, 2022). More recently, a variety of ncAAs have been used to enhance protein catalytic activity or confer new physicochemical properties (Bednar et al., 2023).

Early methods for genetic code expansion often relied on reassigning rare stop codons—especially the amber stop codon (UAG)—to encode ncAAs. Although effective for site-specific incorporation, this approach was limited by competition with the cell's natural translation termination machinery (Shandell et al., 2021). To overcome this, researchers turned to genome-scale engineering, modifying codon usage across entire genomes. It has led to the creation of genomically recoded organisms (GROs), where specific codons are systematically removed or reassigned throughout the genome (Isaacs et al., 2011). These genome-wide recoding strategies demonstrated that large-scale changes to the genetic code are feasible without disrupting essential cellular functions(Lajoie et al., 2013) .

GROs marks a significant milestone in synthetic biology, as they allow deliberate redesign of the genetic code at the organism level. Genome recoding has been successfully applied in microbial systems, particularly in *E. coli*, where targeted codons have been replaced across thousands of genomic sites (Ostrov et al., 2016).This work has culminated in the synthesis of extensively recoded bacterial genomes with reduced codon sets, showing that organisms can thrive even with modified genetic codes (Fredens et al., 2019). Advances in genome engineering now make it possible to build organisms with altered codon usage patterns and better translational control (Ostrov et al., 2020). Codon reassignment has even been used to create organisms with increased resistance to viral infection, highlighting the potential of genome recoding for biocontainment and biosafety (Robertson et al., 2021). Recent improvements in genetic code expansion have significantly enhanced the efficiency and scope of incorporating noncanonical amino acids in biological systems (Dunkelmann & Chin, 2024; Sinha et al., 2023) . This review therefore focuses on the recent advances in the engineering of GROs and explores how these strategies are unlocking new possibilities for designing novel proteins and advanced biomaterials through genetic code expansion. An overview of genetic code expansion and the engineering of genomically recoded organisms is illustrated in Figure 1.

Figure 1. Genetic code expansion and engineering of Genomically recoded organisms (GROs) (Created with https://www.BioRender.com)



Strategies for Generation of Genomically Recoded Organisms

The creation of genomically recoded organisms (GROs) relies on a variety of synthetic biology strategies aimed at reprogramming the genetic code and modifying cellular translation systems. The main goal is to free specific codons from their natural roles and repurpose them to incorporate noncanonical amino acids (ncAAs), thereby expanding the chemical repertoire of proteins. Achieving this typically involves large-scale genome modifications, redesigning translation machinery, and implementing orthogonal molecular systems that operate independently of the cell's native machinery. Advances in genome engineering, paved way to systematically alter codon usage and build organisms with redesigned genetic codes, allowing the production of proteins with new chemical functionalities without compromising cellular viability (Amiram, 2025; James et al., 2025) .Those strategies have enabled more precise manipulation of codon usage and improved flexibility in engineered organisms that genome

is synthetically designed (Costello et al., 2025; Gao et al., 2024; Jann et al., 2024).The major engineering strategies used for the generation of genomically recoded organisms are Summarized in Table 1.

Table 1: Engineering Strategies Used for Generation of GROs

Strategy	Molecular Target	Engineering Approach	Translation Mechanism	Key Outcome	Advantages	Limitations	REFERENCES
Codon reassignment	Stop codons (UAG) or rare codons	Genome-wide codon replacement and elimination of release factor 1	Reassigned codons decoded by engineered tRNA carrying ncAAs	Site-specific incorporation of ncAAs into proteins	Enables efficient site-specific incorporation of ncAAs	Extensive genome engineering required; possible fitness cost	(Fredens et al., 2019; Isaacs et al., 2011)
Orthogonal tRNA–aaRS systems	tRNA and aminoacyl-tRNA synthetase pairs	Directed evolution and rational engineering of aaRS active sites	Orthogonal aaRS selectively charges ncAAs onto engineered tRNA	Specific decoding of reassigned codons	High specificity and modularity across hosts	Potential cross-reactivity with host machinery	(Chin, 2014; Italia et al., 2017)
Ribosome engineering	Ribosomal RNA and ribosomal proteins	Mutagenesis of ribosomal decoding center and peptidyl transferase center	Modified ribosomes accommodate bulky ncAAs	Enhanced translational tolerance for ncAAs	Expands translational tolerance for non-standard substrates	Reduced translation efficiency in some systems	(Hammerling et al., 2020; Orelle et al., 2015)
Quadruplet codon decoding	Codon–anticodon interaction	Engineering tRNAs capable of recognizing four-base codons	Translation machinery decodes expanded codons	Expanded codon capacity beyond the canonical 64 codons	Expands theoretical coding capacity beyond 64 codons	Frameshifting and decoding inefficiency	(Neumann et al., 2010; Tharp et al., 2021)
Synthetic base pairs	DNA nucleobases	Introduction of artificial nucleotides forming orthogonal base pairs	Artificial base pairs generate new codons for ncAA incorporation	Expansion of the genetic alphabet and coding capacity	Dramatically increases coding capacity	Requires engineered replication and transcription systems	(Kimoto & Hirao, 2022; Malyshev et al., 2014)

Early experiments showed that genome-wide codon replacement could be achieved by systematically substituting target codons across the chromosome without affecting essential gene expression (Isaacs et al., 2011) .These pioneering studies proved that rewriting the genetic code at the genomic level is feasible. Subsequent work focused on redesigning microbial genomes to reduce the number of codons used for protein translation, creating new opportunities for codon reassignment and ncAAs incorporation (Ostrov et al., 2016). More recent developments in synthetic genome construction and large-scale DNA assembly have accelerated GRO generation and broadened the range of genome engineering strategies available (Zürcher et al., 2023).

Stop Codon Reassignment Strategies

Reassigning the stop codons to incorporate noncanonical amino acids (ncAAs) into proteins has remained a key strategy for expanding the functionalities of genetic code. In the standard genetic code, the three stop codons—UAA, UAG, and UGA—signal the end of translation. Among these, the amber codon (UAG) is used least frequently, making it an ideal candidate for reassignment (Young & Schultz, 2010).By systematically replacing all native UAG codons in the genome and removing the release factor that recognizes this codon, researchers can free it up for precise, site-specific incorporation of ncAAs (Lajoie et al., 2013).

This approach relies on engineered orthogonal tRNA–aminoacyl-tRNA synthetase (aaRS) pairs that operate independently from the cell's natural translation machinery. These orthogonal pairs ensure that ncAAs are inserted accurately at the reassigned codon without disrupting normal protein synthesis (Chin, 2017). Advances in orthogonal system design have greatly improved both the efficiency and fidelity of codon reassignment, and a

variety of tRNA–aaRS combinations are now available to accommodate ncAAs with diverse chemical properties (Aleksashin et al., 2020; Andrews et al., 2023) .

Experimental studies have validated the effectiveness of this strategy. For instance, in *E. coli*, removing all native UAG codons allowed the amber codon to be exclusively used for ncAAs incorporation(Lajoie et al., 2013). Later genome engineering efforts produced strains with simplified codon usage and greater translational flexibility (Fredens et al., 2019). A recent work has even generated strains using only a single stop codon, freeing additional codons for reassignment (Grome et al., 2025).

Beyond expanding coding potential, stop codon reassignment also enhances biosafety. By making engineered organisms dependent on externally supplied ncAAs, their survival is restricted to controlled laboratory conditions (Rovner et al., 2015).Furthermore, codon reassignment can reduce viral susceptibility by preventing viruses from hijacking the host's translation machinery (Nyerges et al., 2023) .Improved codon reassignment approaches have enhanced translational efficiency while minimizing interference from endogenous release factors (Tharp et al., 2021) .Together, these features make stop codon reassignment a powerful tool in synthetic biology-based biocontainment strategies.

Sense Codon Compression

While stop codon reassignment is a foundational approach, more extensive genome recoding can be achieved through sense codon compression. This strategy reduces redundancy in the genetic code by removing selected synonymous codons and replacing them with alternative codons that encode the same amino acid. By compressing codon usage across the genome, additional codons become available for reassignment to ncAAs (Robertson et al., 2021).

Sense codon compression requires precise, large-scale genome redesign. Advances in computational genome design and DNA synthesis have enabled systematic rewriting of microbial genomes to reduce codon redundancy (Ostrov et al., 2016) .These efforts have produced bacterial strains with simplified genetic codes containing fewer functional codons while maintaining viability and metabolic function (Fredens et al., 2019).

Extensive codon compression has also allowed the simultaneous incorporation of multiple ncAAs within a single organism, greatly expanding the chemical diversity possible through translation (Dunkelmann et al., 2021) .Additionally, sense codon reassignment has enabled the production of novel polymers and biomolecules with properties not found in nature, highlighting codon compression as a powerful tool for expanding the functional capabilities of synthetic organisms (Robertson et al., 2021) .Codon compression strategies have demonstrated the potential to expand coding capacity while maintaining cellular functionality (Pagar et al., 2022).

Genome Engineering Platforms

Generating GROs relies on advanced genome engineering technologies capable of large-scale DNA modifications. Key platforms include multiplex automated genome engineering (MAGE), conjugative assembly genome engineering (CAGE), and CRISPR-based editing systems, all of which enable precise and scalable genome redesign (Isaacs et al., 2011).

MAGE allows simultaneous modification of multiple genomic sites through iterative cycles of oligonucleotide-mediated recombination, making it ideal for targeted codon replacement across bacterial genomes (Lajoie et al., 2013) .CAGE further enhances this by enabling hierarchical assembly of modified genomic segments into a single recoded chromosome (Ostrov et al., 2016). Together, these platforms provide robust tools for large-scale genome redesign.

CRISPR-based systems have added another level of precision and flexibility. They enable efficient, targeted editing of genomic sequences, facilitating codon replacement and gene modification at unprecedented scales (Xu et al., 2023). Combining CRISPR editing with synthetic genome assembly strategies has made it possible to construct organisms with fully redesigned genetic codes (James et al., 2025) .

Beyond genome editing, modifying translation machinery is also critical. Engineering ribosomes, tRNAs, and aminoacyl-tRNA synthetases can improve the efficiency and fidelity of ncAAs incorporation (Ishida et al., 2024). Specialized ribosomes with altered decoding properties have been developed to support unconventional amino acids and expanded codon systems (Carlson et al., 2019). Together, these advances have greatly expanded the toolkit for building genomically recoded organisms. The New developments in large-scale genome engineering have enabled efficient and programmable modification of genetic systems (Y. Chen et al., 2023; Z. Wu & Wang, 2023).

Orthogonal Translation Systems in GROs

Orthogonal translation systems (OTSs) are at the heart of genomically recoded organisms (GROs) because they allow the incorporation of noncanonical amino acids (ncAAs) without interfering with the host's normal protein synthesis. The idea of orthogonality in translation originated from efforts to introduce engineered tRNA -aaRs pairs that specifically recognize noncanonical substrates while remaining functionally isolated from endogenous translation systems. OTSs comprise engineered translational components—mainly tRNAs, aminoacyl-tRNA synthetases (aaRSs), and sometimes ribosomes—that operate independently of the cell's native machinery. The development of OTSs has been key to expanding the genetic code, enabling the precise insertion of chemically diverse amino acids into proteins. By isolating engineered translational elements from the host's translation

network, orthogonal systems facilitate codon reassignment and the production of proteins with novel biochemical functionalities (Chin, 2017). Early work demonstrated that orthogonal components from archaeal organisms could function in heterologous hosts, selectively incorporating ncAAs at reassigned codons (Chin et al., 2003). Since then, orthogonal translation technologies have advanced significantly, enabling the incorporation of many synthetic amino acids and supporting sophisticated applications in protein engineering and synthetic biology (Shandell et al., 2021). In GROs, orthogonal systems are particularly valuable because genome recoding can create unused codons that serve as dedicated entry points for ncAA incorporation(Lajoie et al., 2013). The achievements in the genome recoding and synthetic biology have enhanced the compatibility between GROs and orthogonal platforms, allowing more effective and scalable genetic code expansion (De La Torre & Chin, 2021) .Advances in orthogonal translation components have significantly improved the specificity and efficiency of noncanonical amino acid incorporation (Zürcher et al., 2025).

Principles underlying Orthogonality

In the context of genetic code expansion, orthogonality refers to the ability of engineered translational components to function independently of the host's natural protein synthesis machinery. In a fully orthogonal system, an engineered tRNA is recognized exclusively by its matching engineered aminoacyl-tRNA synthetase (aaRS), while the cell's native synthetases do not interact with it. The engineered aminoacyl-tRNA synthetase specifically attaches the target non-canonical amino acid to its corresponding orthogonal tRNA, ensuring it does not interact with the cell's natural tRNAs. This precise pairing ensures that ncAAs are incorporated only at targeted codons, without disrupting the cell's normal protein translation. OTSs have been successfully applied in both prokaryotic and eukaryotic organisms. Among the most widely used are archaeal pyrrolysyl-tRNA synthetase/tRNA pairs, which are highly adaptable and can be engineered to accept a broad range of synthetic amino acids (Koch & Budisa, 2024; Lang & Chin, 2014) .Using these systems, researchers have been able to site-specifically incorporate hundreds of ncAAs with diverse chemical functionalities into proteins(Huang et al., 2025).

The concept of orthogonality has also been extended to larger translational components. For example, modified ribosomes have been designed to operate independently of the cell's native ribosomes, creating specialized translation pathways for synthetic genetic codes (Carlson et al., 2019) .These orthogonal ribosomes can efficiently decode reassigned codons and translate engineered mRNAs, significantly improving the incorporation of ncAAs in recoded organisms (Jia et al., 2017).

Engineering tRNA–Aminoacyl-tRNA Synthetase Pairs

Designing orthogonal tRNA–aaRS pairs is crucial for genetic code expansion. These pairs are engineered to recognize specific ncAAs while maintaining strict specificity for their corresponding tRNAs. Directed evolution is commonly used to modify the aaRS substrate-binding pocket, improving its affinity for synthetic amino acids (Chin, 2014) .Iterative selection and screening have produced many synthetases capable of charging orthogonal tRNAs with a wide range of ncAAs. The pyrrolysyl-tRNA synthetase/tRNA system, first discovered in methanogenic archaea, is generally used due to its moderate substrate specificity and modular structure, allowing it to adopt numerous ncAAs (Koch & Budisa, 2024). Other orthogonal tRNA–aaRS pairs have also been developed to expand ncAA diversity and improve host compatibility (Andrews et al., 2023) .The tRNA engineering has further improved OTS performance. Structural modifications enhance decoding efficiency, codon recognition, and reduce misincorporation (Y. Kim et al., 2024). Rational design has also enabled incorporation of D-amino acids and other unconventional substrates, significantly broadening the range of proteins accessible via genetic code expansion (Jiang et al., 2023).

Multisite Incorporation of Noncanonical Amino Acids

A major goal in genetic code expansion is the ability to incorporate multiple noncanonical amino acids (ncAAs) at specific positions within a single protein. Introducing several ncAAs allows proteins to carry a range of chemical functionalities, which can enhance their catalytic activity, structural stability, or regulatory roles. As stated earlier, early research on genetic code expansion introduced a single non-canonical amino acid at specific sites by employing stop codon suppression through orthogonal translation systems. Expanding this approach to multiple ncAAs, however, requires more sophisticated engineering of the translational machinery.

One strategy uses several mutually orthogonal tRNA–aminoacyl-tRNA synthetase (aaRS) pairs, each designed to recognize a distinct codon, enabling different ncAAs to be inserted at defined positions (Italia et al., 2019) .Another approach relies on genome recoding to create additional unused codons, providing new "slots" for ncAA incorporation (Robertson et al., 2021) .By combining these strategies, researchers can precisely control the placement of multiple synthetic amino acids within a protein.

Cell-free protein synthesis systems derived from genomically recoded organisms (GROs) further enhance multisite ncAA incorporation by providing a controlled, cell-independent environment that avoids metabolic limitations and improves efficiency (Des Soye et al., 2019; Martin et al., 2018) .Recent advances integrating automated orthogonal translation systems with redesigned mRNAs have made it possible to incorporate up to four distinct ncAAs into a single protein (Dunkelmann et al., 2021) ,demonstrating the growing potential to engineer proteins with highly complex and customizable chemical properties.

Efficiency and Fidelity Optimization

Despite these advances, achieving high efficiency and fidelity in ncAA incorporation remains challenging. Factors such as inefficient aminoacylation, competition with endogenous translation machinery, and misincorporation of canonical amino acids can reduce accuracy. Consequently, extensive efforts have focused on optimizing orthogonal translation systems (Fu et al., 2022).

Strategies include directed evolution of aaRSs to enhance substrate specificity and catalytic activity, improving tRNA charging efficiency (Lino et al., 2024).Ribosomal engineering also improves decoding accuracy and reduces competition between orthogonal and native translation pathways (Ishida et al., 2024).

Alternative codon systems provide additional improvements. Quadruplet codon decoding makes extra codons beyond the canonical triplet system, expanding coding capacity (Guo & Niu, 2022) . Simplified codon sets in engineered genomes also support more reliable ncAA incorporation (Ostrov et al., 2016) .

Together, these optimization strategies have greatly increased the efficiency and reliability of OTSs in GROs. Continued refinement of translational components, combined with advances in genome engineering and synthetic biology, is expected to further enhance the ability of GROs to produce proteins containing diverse noncanonical amino acids.

GROs for Novel Protein Engineering

Genomically recoded organisms (GROs) have become powerful platforms for creating proteins with expanded chemical functionality. By redesigning the genetic code and incorporating noncanonical amino acids (ncAAs), GROs allows the production of proteins carrying chemical groups that do not exist in the standard set of twenty amino acids. The incorporation of noncanonical amino acids has facilitated the development of proteins with enhanced structural and functional diversity (Illies et al., 2025; Katoh & Suga, 2024; K. Wu et al., 2022).These modifications provide precise control over protein structure, function, and reactivity, opening up new opportunities in biotechnology, therapeutics, and the development of advanced biomaterials (Chin, 2017; De La Torre & Chin, 2021) .In conventional biological systems, protein synthesis is limited by the standard genetic code, which restricts the diversity of chemical modifications that can be introduced. By combining genetic code expansion with genome recoding, GROs overcomes these limitations, freeing codons for reassignment to ncAAs and enabling the synthesis of proteins with novel chemical and physical properties (Mukai et al., 2017).

GRO-based protein engineering relies on the coordinated integration of genome recoding, orthogonal translation machinery, and efficient ncAAs incorporation systems. Together, these technologies allow precise insertion of chemically reactive residues into proteins, supporting site-specific functionalization, multisite modification, and the creation of proteins with enhanced catalytic or structural properties (Shandell et al., 2021; Z. Wu & Wang, 2023). Moreover, genome-wide codon reassignment reduces competition with natural translation components, improving the efficiency of ncAA incorporation. This makes GROs a versatile platform for designing proteins with novel functions that are difficult or impossible to achieve using traditional protein engineering methods (Young & Schultz, 2010) .

Site-Specific Chemical Functionalization

The capacity of GROs to add site-specific chemical functions to proteins is one of their most significant uses. Through genetic code expansion, unique reactive groups—such as azides, alkynes, or ketones—can be incorporated at defined positions within a protein sequence. These chemically reactive amino acids allow proteins to undergo selective bioorthogonal reactions, facilitating the targeted attachment of fluorescent labels, polymers, or therapeutic compounds (J. Chen & Tsai, 2022; Lang & Chin, 2014).

Site-specific labeling using ncAAs has become an essential tool for studying protein dynamics and interactions in living cells. Engineered orthogonal translation systems can insert ncAAs at predetermined codons, enabling proteins to be modified with imaging probes or chemical cross-linkers without altering their native structure (Bednar et al., 2023) .This approach has facilitated detailed investigations of protein folding, conformational changes, and post-translational modifications.

Beyond analytical applications, site-specific functionalization also enables the development of protein-based therapeutics with improved pharmacological properties. Incorporating reactive amino acids into proteins enables their attachment to polymers such as polyethylene glycol, which can improve protein stability and solubility and extend their circulation duration in living systems (Liu & Schultz, 2010) .Such strategies highlight how GROs can be harnessed to design next-generation protein therapeutics with controllable chemical modifications and improved performance.

Multi-ncAA Incorporation

GROs also allow the simultaneous incorporation of multiple ncAAs into a single protein, greatly expanding the chemical complexity of proteins and enabling the creation of sophisticated molecular architectures (Schmied et al., 2014). Achieving this requires highly efficient orthogonal translation systems and engineered ribosomes capable of decoding reassigned codons without interfering with native translation.

Recent advances have demonstrated the feasibility of incorporating several distinct ncAAs by using different codon reassignments or orthogonal translation components. For example, synthetic genetic codes with additional codons enable multiple chemically distinct residues to be incorporated within a single polypeptide chain (Dunkelmann et al., 2021) .Similarly, mutually orthogonal tRNA–aaRS pairs allow independent decoding of

multiple codons, facilitating the site-specific incorporation of different ncAAs in the same protein molecule (Italia et al., 2019).

Cell-free expression systems derived from GROs further enhance multisite ncAA incorporation by removing cellular constraints on protein synthesis. Using these platforms, researchers have successfully introduced multiple ncAAs at defined positions, generating proteins with complex structures and novel functionalities (Martin et al., 2018) .These approaches illustrate the power of GROs as a robust platform for engineering proteins with unprecedented chemical diversity.

Enhanced Catalytic and Structural Properties

Incorporating ncAAs also enables the creation of enzymes and structural proteins with enhanced catalytic or physicochemical properties. Because ncAAs can carry functional groups not present in natural amino acids, they can introduce new catalytic mechanisms or improve substrate interactions in engineered enzymes (Zhao et al., 2020) .Such modifications can dramatically enhance enzyme performance in industrial biocatalysis and synthetic chemistry.

For instance, adding reactive side chains can generate new catalytic centers that facilitate reactions not normally catalyzed by natural enzymes.

Research has demonstrated that in harsh circumstances, enzymes containing ncAAs may display improved stability, changed substrate selectivity, or greater catalytic performance (Li & Dalby, 2022).These improvements make ncAA-containing enzymes attractive for applications in biotechnology, pharmaceutical synthesis, and environmental biocatalysis.

Structural proteins also benefit from ncAA incorporation. Novel residues can introduce cross-linking capabilities, enhance mechanical strength, or modify folding dynamics, enabling the design of advanced protein-based materials with improved functional properties (Birch-Price et al., 2024). GRO-mediated protein engineering therefore offers a promising route for creating biomolecules with tailored structural and functional characteristics.

Viral Resistance and Proteome Isolation

Another key advantage of GROs is intrinsic resistance to viral infections and horizontal gene transfer. By recoding an organism's genetic code, specific codons can be reassigned to encode ncAAs instead of their natural amino acids. Viruses relying on the standard genetic code cannot properly translate their proteins in such hosts, effectively preventing viral replication (Robertson et al., 2021).

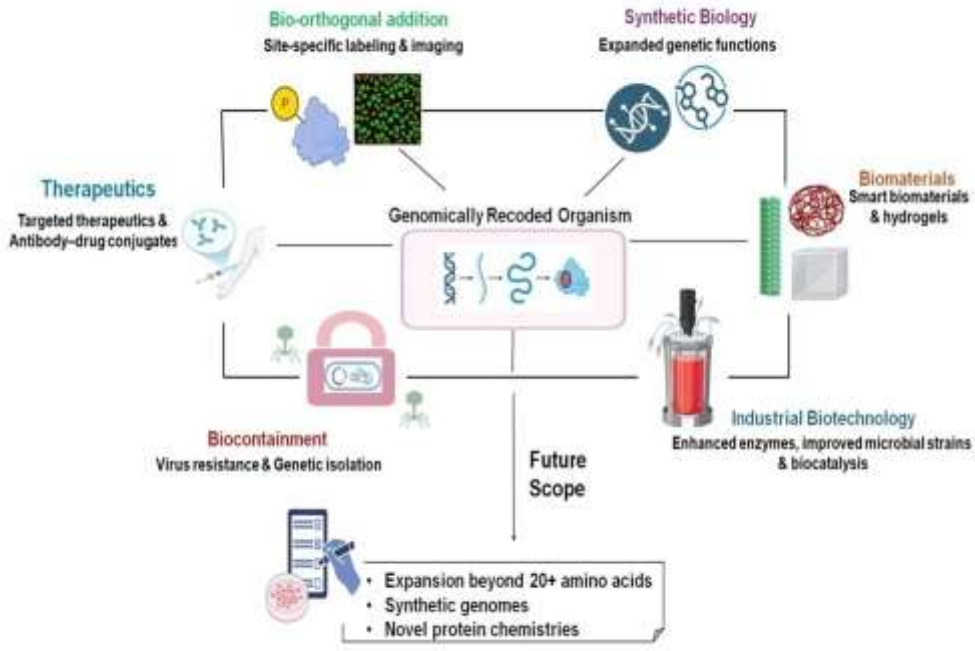
Studies have shown that organisms with extensively recoded genomes can resist a wide range of bacteriophages due to incompatibilities between viral genetic codes and the host translation machinery. This provides a powerful strategy for creating genetically isolated organisms that are protected from viral contamination in industrial or laboratory environments (Nyerges et al., 2023). Furthermore, genome-wide recoding enables reassignment of multiple codons, strengthening the "genetic firewall" between engineered organisms and natural biological systems.

Proteome isolation is another outcome of large-scale genome recoding. In GROs, reassigned codons can be dedicated exclusively to ncAAs incorporation, allowing engineered proteins to be produced selectively while minimally affecting native cellular processes (Perez et al., 2022) .This controlled environment is particularly valuable for large-scale biotechnological applications, ensuring stability, biosafety, and protection against biological contamination.

Applications in Biomaterial Engineering

Recent advances in genomically recoded organisms (GROs) and genetic code expansion technologies have opened up exciting possibilities for designing biomaterials with precisely tunable structural and functional properties (K. Wu et al., 2022). By incorporating noncanonical amino acids (ncAAs) into proteins, GRO-based systems provide a platform for creating biomaterials with chemical functionalities not found in natural proteins. These engineered proteins can be tailored for a wide range of applications, including tissue engineering, drug delivery, biosensing, and smart materials (Chin, 2017; De La Torre & Chin, 2021).The ability to introduce unique chemical groups allows precise control over crosslinking, assembly, and responsiveness, greatly expanding the possibilities of synthetic biology in materials science (Shandell et al., 2021; H. Tang et al., 2022) .The diverse applications of genomically recoded organisms and genetic code expansion in therapeutics, biomaterials, and synthetic biology are summarized in Figure 2.

Figure 2. Applications and future scope of GRO Engineering (Created with https://www.BioRender.com)



Functional Protein-Based Hydrogels

Protein-based hydrogels are a key class of biomaterials, capable of mimicking the structural and mechanical properties of natural tissues. Incorporating ncAAs into hydrogel-forming proteins enables the addition of bio-orthogonal reactive groups, allowing controlled polymerization and crosslinking. GRO-derived proteins are particularly useful for designing hydrogels with programmable characteristics. Site-specific incorporation of ncAAs allows for hydrogels with improved biocompatibility, alterable elasticity, and enhanced structural stability (Birch-Price et al., 2024) .Cell-free protein synthesis systems derived from GROs further facilitate rapid production of hydrogel-forming proteins with multiple ncAAs, supporting advanced biomaterial design for applications such as tissue regeneration and cell growth (Choi et al., 2023; Martin et al., 2018).

Self-Assembling Protein Polymers

GRO technology also enables the development of self-assembling protein polymers. These proteins can spontaneously organize into ordered nanostructures—such as fibers, sheets, or nanoparticles—that are valuable for biomedical and nanotechnological applications. Researchers can precisely control intermolecular interactions and direct the self-assembly process by incorporating ncAAs into protein monomers (Liu & Schultz, 2010) .

Expanding the genetic code allows functional groups to be strategically placed, promoting selective interactions between protein subunits and generating highly stable polymer networks with improved catalytic or structural properties (Li & Dalby, 2022) .Incorporating multiple distinct ncAAs into the same protein provides additional flexibility to create multifunctional polymers with tunable chemical and mechanical characteristics (Dunkelmann et al., 2021; McFeely et al., 2023).

GROs also enables large-scale production of these protein polymers. Genome recoding reduces competition between engineered translation components and the host machinery, allowing efficient ncAAs incorporation at multiple sites and supporting the construction of complex, functional biomaterials (Perez et al., 2022).

Click Chemistry-Enabled Crosslinking

Click chemistry has emerged as a key tool in biomaterial engineering because it allows rapid and highly specific chemical reactions under mild conditions. Genetic code expansion enables the site-specific introduction of these reactive groups, supporting the design of biomaterials with controlled architectures. Orthogonal translation systems can introduce ncAAs that participate in copper-catalyzed or strain-promoted azide–alkyne cycloaddition reactions, forming stable, crosslinked networks (Italia et al., 2019). Proteins containing multiple reactive residues can even undergo sequential click reactions, producing hierarchically organized biomaterials with complex structures (Bednar et al., 2023; L. Chen et al., 2023) .These strategies are particularly valuable for targeted drug delivery, tissue scaffolding, and other biomedical applications**.**

Stimuli-Responsive Biomaterials

Stimuli-responsive biomaterials are designed to change their physical or chemical properties in response to environmental cues, such as temperature, pH, light, or chemical signals. Incorporating ncAAs provides a powerful approach to engineer such responsiveness, as these amino acids can introduce chemically sensitive functional groups that respond to specific triggers (Huang et al., 2025).

For instance, proteins engineered with photocaged or redox-active ncAAs can undergo conformational changes in response to light or oxidative conditions, creating "smart" biomaterials with controlled activation (Katoh & Suga, 2022). Similarly, ncAA-containing proteins can form responsive polymer networks that dynamically adjust their mechanical properties based on environmental changes (D. Kim et al., 2025) .

GRO-based systems also support the development of living biomaterials, where engineered microbes embedded within biomaterial scaffolds can sense environmental cues or perform computational functions. Hydrogel matrices containing engineered bacteria have demonstrated the ability to respond to stimuli while remaining physically contained, highlighting the potential of GRO-enabled protein engineering for next-generation responsive biomaterials (T.-C. Tang et al., 2021). The key applications of GRO-derived proteins in biomaterial and therapeutic engineering are summarized in Table 2.

Table 2: Applications of GRO-Derived Proteins in Biomaterial and Therapeutic Engineering

Application	Functional role	Key Features	References
Functional Protein-Based Hydrogels	Hydrogels formed from ncAA-containing proteins	Tunable elasticity, biocompatibility, programmable crosslinking	(Birch-Price et al., 2024; Choi et al., 2023; Martin et al., 2018)
Self-Assembling Protein Polymers	Proteins self-organize into fibers, sheets, or nanoparticles	Structural stability, customizable interactions, multifunctional	(Dunkelmann et al., 2021; Li & Dalby, 2022; Liu & Schultz, 2010)
Click Chemistry-Enabled Crosslinking	ncAA residues (azides/alkynes) allow bioorthogonal crosslinking	Hierarchical network formation, precise chemical conjugation	(Bednar et al., 2023; L. Chen et al., 2023; Italia et al., 2019)
Stimuli-Responsive Biomaterials	Proteins respond to environmental cues (light, pH, redox)	Smart biomaterials, dynamic mechanical properties, living biomaterials	(Huang et al., 2025; Katoh & Suga, 2022; D. Kim et al., 2025)
Therapeutic Protein Engineering	ncAA incorporation for PEGylation or drug conjugation	Enhanced solubility, stability, and in vivo circulation	(J. Chen & Tsai, 2022; Lang & Chin, 2014; Liu & Schultz, 2010)

Biosafety and Genetic Firewalls

The rapid progress in genomically recoded organisms (GROs) and genetic code expansion has raised important considerations regarding biosafety, environmental containment, and the prevention of unintended genetic exchange with natural organisms. Because GROs involves extensive genome modifications and the use of noncanonical amino acids (ncAAs), it is crucial to implement robust safety mechanisms to ensure these engineered organisms remain contained and do not disrupt natural ecosystems. To address these concerns, researchers have developed strategies collectively known as genetic firewalls, which limit the ability of synthetic organisms to survive or exchange genetic material with natural biological systems (Mandell et al., 2015).These strategies rely on genome recoding, engineered metabolic dependencies, and translational incompatibilities that prevent horizontal gene transfer and viral infections (Nyerges et al., 2023).As GRO technologies advance, designing reliable biosafety frameworks has become an integral part of synthetic biology research .Genetic code modifications have been explored as a strategy to improve biological containment and reduce unintended gene transfer (Hammerling et al., 2020) .

Synthetic Auxotrophy

One of the most widely adopted approaches for biocontainment is synthetic auxotrophy. In this strategy, engineered organisms are made dependent on synthetic molecules that do not exist in natural environments. The organism can only survive and grow when these compounds are supplied in controlled laboratory or industrial settings (Rovner et al., 2015) .Without the synthetic molecule, essential biological processes cannot proceed, and the organism fails to survive outside the designed environment.

A particularly effective approach involves reassigning codons to encode ncAAs. Essential proteins are engineered to include these ncAAs, which must be provided externally for proper protein synthesis. If the organism escapes into the wild, where the ncAAs is unavailable, essential protein production halts, preventing cell growth and replication (Mandell et al., 2015).This approach has been successfully implemented in recoded *E.coli* strains, demonstrating a reliable mechanism for biological containment (Lajoie et al., 2013).

Genome recoding further strengthens synthetic auxotrophy by enabling systematic codon reassignment across multiple essential genes. Integrating ncAA dependence into several key proteins reduces the likelihood of evolutionary escape, creating organisms with built-in safety mechanisms (Fredens et al., 2019) .

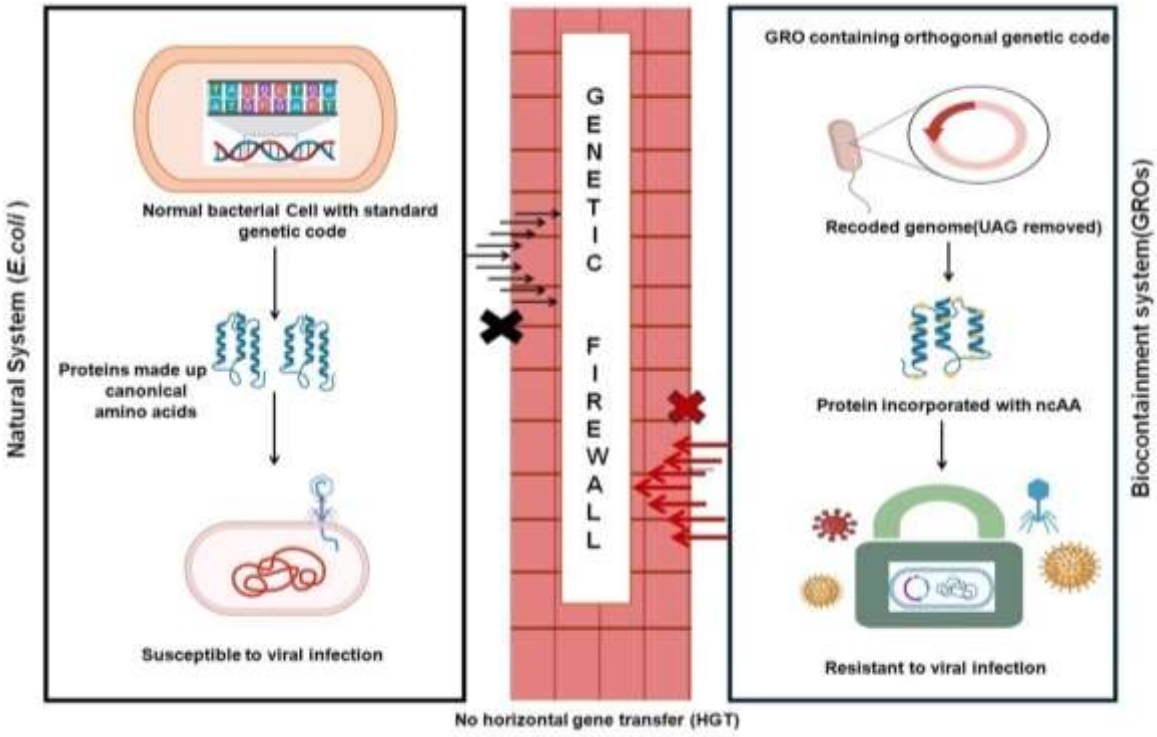
Genetic Firewall Concept

A genetic firewall is a biological barrier that prevents the exchange of genetic information between engineered and natural organisms. In traditional genetic engineering, horizontally transferred genes may still function in natural hosts because they use the same universal genetic code. However, genome recoding can alter codon usage or translational machinery, making genes incompatible with natural organisms (Kuo et al., 2018) .

For example, recoded organisms may use modified codons or orthogonal translation components. Genes transferred from these engineered organisms may not be translated correctly in wild-type hosts, and natural genes may fail in the recoded host (Ostrov et al., 2016). These incompatibilities create a barrier that effectively limits gene flow. The concept of a genetic firewall and its role in preventing horizontal gene transfer and viral propagation between natural and engineered systems is illustrated in Figure 3.

Orthogonal translation systems further reinforce genetic firewalls. By engineering ribosomes and tRNA–synthetase pairs that function independently from native translation machinery, researchers can establish synthetic translational pathways that are inaccessible to natural organisms, ensuring that synthetic genetic codes remain isolated (Jia et al., 2017).

Figure 3. Concept of a Genetic firewall for gene transfer between natural and engineered GRO systems (Created with https://www.BioRender.com)



Viral Resistance Through Codon Reassignment

GROs also offers a natural resistance to viral infections. Many viruses rely on the host's translational machinery to produce viral proteins. When the host's genetic code is recoded, viral genes may no longer be translated properly, preventing viral replication (Robertson et al., 2021).

Large-scale genome recoding has shown that organisms with modified genetic codes can resist a wide range of bacteriophages. By reassigning specific codons to encode ncAAs, translation of viral proteins is disrupted, providing a built-in protective mechanism for engineered microbial strains used in industrial or laboratory applications(Nyerges et al., 2023). Codon compression and reassignment strategies can further enhance viral resistance by eliminating codons commonly used by viral genomes (Grome et al., 2025)

Environmental and Regulatory Considerations

Despite these advanced containment strategies, the potential environmental release of engineered organisms remains a key regulatory concern. Applications of GROs must comply with strict biosafety guidelines and undergo thorough risk assessments to evaluate ecological impact (James et al., 2025).

Risk assessments typically examine genetic stability, potential for horizontal gene transfer, and the likelihood of survival outside controlled environments. Techniques such as genome minimization, codon reassignment, and synthetic auxotrophy reduce these risks by limiting compatibility with natural ecosystems (Xu et al.,

2023).Furthermore, modern genome design allows multiple biosafety mechanisms to be integrated within a single organism, improving containment reliability.

As synthetic biology continues to evolve, international regulatory frameworks will play a critical role in guiding the safe development and deployment of GRO technologies. Collaborative efforts between researchers, policymakers, and regulatory agencies are essential to balance innovation with responsible environmental stewardship (Amiram, 2025) .

Challenges, Gaps, and Limitations of GRO Engineering

Despite remarkable advances in genome recoding and genetic code expansion, several challenges continue to limit the broader implementation of genomically recoded organisms (GROs). One of the main hurdles is the complexity of large-scale genome engineering. Recoding an entire genome requires extensive codon replacements and precise chromosome editing, which can unintentionally affect gene regulation, protein expression, and overall cellular fitness. Early studies showed that even a large number of synonymous codon changes can disrupt translation efficiency and cell physiology, making it difficult for extensively recoded organisms to grow robustly (Isaacs et al., 2011; Martínez et al., 2019) . Even in advanced systems like fully recoded *Escherichia coli*, adaptive evolution was often necessary to restore optimal growth and metabolic function (Fredens et al., 2019; Wannier et al., 2018).

Another significant challenge lies in the efficiency and fidelity of orthogonal translation systems (OTSs) used to incorporate noncanonical amino acids (ncAAs). Orthogonal tRNA–aminoacyl-tRNA synthetase (aaRS) pairs must operate independently of the host's translation machinery, yet achieving both strict orthogonality and high incorporation efficiency is difficult. Many engineered systems still show incomplete suppression, misincorporation of canonical amino acids, or competition with native translation components (Aleksashin et al., 2020; Fu et al., 2022) .In addition, the number of fully orthogonal tRNA–aaRS pairs capable of recognizing diverse ncAAs is still limited, restricting the range of chemical modifications that can be introduced into proteins (Andrews et al., 2023; Y. Kim et al., 2024).

Increasing the number of codons available for ncAA incorporation also presents practical hurdles. Strategies such as quadruplet codon decoding, unnatural base pairs, and codon compression can expand coding capacity but often suffer from low decoding efficiency and reduced translational accuracy (Guo & Niu, 2022; Neumann et al., 2010). Similarly, approaches involving semisynthetic organisms or expanded genetic alphabets add complexity to DNA replication and cellular stability, making stable inheritance over multiple generations a persistent challenge (Malyshev et al., 2014; Zhang et al., 2017).

The metabolic burden of ncAA incorporation is another limitation. Many ncAAs are not naturally produced by host cells and must be supplied externally or synthesized via engineered metabolic pathways. This can increase production costs and limit scalability in industrial applications (Birch-Price et al., 2024; Y. Chen et al., 2023).Incorporating multiple ncAAs within a single protein can further reduce translation efficiency and protein yields, especially in large or complex proteins (Bednar et al., 2023).

Finally, biosafety and regulatory concerns remain critical. While genetic firewalls and synthetic auxotrophy can mitigate the risk of gene transfer or environmental survival, the long-term ecological impact of releasing engineered organisms is still uncertain(Mandell et al., 2015; Nyerges et al., 2023). Overcoming these challenges will require continued innovation in genome engineering, translation system optimization, and safety design. Addressing these gaps is essential for realizing the full potential of GROs in biotechnology, medicine, and biomaterials engineering (Amiram, 2025).

Future Perspectives

The field of genomically recoded organisms (GROs) is advancing rapidly and is expected to play a transformative role in synthetic biology, protein engineering, and biomaterials research. Recent progress in genome-scale engineering, synthetic genome construction, and genetic code expansion has demonstrated that extensive rewriting of the genetic code is feasible while maintaining cellular viability and functionality (Amiram, 2025; James et al., 2025) .Future developments are likely to focus on expanding the number of codons available for reassignment, thereby enabling the incorporation of a broader repertoire of noncanonical amino acids (ncAAs) with diverse chemical functionalities. Emerging approaches involving quadruplet codons, expanded genetic alphabets, and engineered translational systems may significantly increase coding capacity beyond the constraints of the canonical genetic code (Guo & Niu, 2022; Kimoto & Hirao, 2022) .

Continued improvements in orthogonal translation systems are expected to enhance the efficiency, fidelity, and scalability of ncAA incorporation. Advances in tRNA engineering, aminoacyl-tRNA synthetase evolution, and ribosome engineering may facilitate the simultaneous incorporation of multiple distinct ncAAs into a single protein with high precision (Ishida et al., 2024; Y. Kim et al., 2024). Such capabilities will enable the design of proteins with unprecedented structural complexity and functional diversity, supporting the development of novel biocatalysts, therapeutic proteins, biosensors, and molecular devices (Chin et al., 2003).In addition, recent studies have demonstrated the feasibility of incorporating multiple ncAAs through automated orthogonal translation systems, suggesting that increasingly sophisticated protein architectures may soon become accessible for industrial and biomedical applications (Dunkelmann et al., 2021) .

Future GRO platforms are also expected to accelerate innovation in biomaterial engineering. The integration of genetic code expansion with programmable protein design could facilitate the development of next-generation hydrogels, self-assembling protein polymers, and stimuli-responsive biomaterials with precisely tunable mechanical and biochemical properties (Birch-Price et al., 2024; Huang et al., 2025).The incorporation of bioorthogonal functional groups into engineered proteins may further enable the construction of smart biomaterials capable of controlled drug release, tissue regeneration, environmental sensing, and adaptive responses to external stimuli. Coupled with advances in cell-free protein synthesis systems derived from recoded organisms, these technologies may provide scalable platforms for manufacturing complex protein-based materials with high precision and reproducibility (Des Soye et al., 2019; Martin et al., 2018) .

Another promising direction involves the development of genetically isolated biological systems with enhanced biosafety features. Recent demonstrations of viral resistance through codon reassignment and genetic code locking have highlighted the potential of GROs to establish robust genetic firewalls against horizontal gene transfer and viral infection(Nyerges et al., 2023; Zürcher et al., 2025) .Future research will likely focus on integrating multiple containment strategies, including synthetic auxotrophy, genome minimization, and orthogonal translation pathways, to improve the long-term stability and environmental safety of engineered organisms (Rovner et al., 2015; Xu et al., 2023).Such developments will be particularly important for the deployment of GRO-based technologies in industrial biomanufacturing, environmental biotechnology, and therapeutic applications.

The integration of artificial intelligence (AI) and machine learning with synthetic biology is expected to further accelerate the development of genomically recoded organisms. AI-driven approaches can assist in predicting codon optimization strategies, designing orthogonal translation components, engineering aminoacyl-tRNA synthetases with improved substrate specificity, and identifying novel noncanonical amino acids with desirable physicochemical properties. Furthermore, computational genome design platforms may facilitate large-scale genome recoding while minimizing adverse effects on cellular fitness and gene regulation. The combination of AI-guided design, automated DNA synthesis, and high-throughput screening technologies could significantly reduce the time and cost associated with constructing next-generation GROs, thereby enabling more efficient development of engineered biological systems for industrial, biomedical, and environmental applications (Costello et al., 2025; Lino et al., 2024).

Despite these advances, several challenges remain, including the metabolic burden associated with ncAA utilization, evolutionary instability of recoded genomes, and the high costs associated with large-scale genome engineering. Addressing these limitations will require continued innovation in synthetic genome design, computational biology, metabolic engineering, and automation technologies (Gao et al., 2024; James et al., 2025).As these challenges are overcome, GROs is expected to emerge as versatile platforms for creating tailor-made biological systems with expanded chemical capabilities, opening new frontiers in biotechnology, medicine, and advanced biomaterials engineering.

CONCLUSION

Genomically recoded organisms (GROs) represent a major advancement in synthetic biology by enabling systematic modification of the genetic code to expand the functional capabilities of living systems. Through genome-wide codon reassignment and the development of orthogonal translation systems, GROs allows the incorporation of noncanonical amino acids into proteins, thereby significantly increasing the chemical diversity available for protein engineering. These developments have opened new possibilities for designing proteins with enhanced catalytic activity, improved structural stability, and novel chemical functionalities that extend beyond the limitations of the canonical genetic code.

The integration of genetic code expansion technologies with genome engineering platforms has also created promising opportunities for biomaterial development. Engineered proteins containing noncanonical amino acids can be designed to form functional hydrogels, self-assembling protein polymers, and responsive biomaterials with applications in tissue engineering, drug delivery, and advanced biomedical materials. In addition, genome recoding strategies provide important biosafety advantages by enabling genetic firewalls and viral resistance mechanisms that improve the stability and containment of engineered organisms.

Future progress in GRO engineering will depend on improving the efficiency of orthogonal translation systems, expanding the repertoire of noncanonical amino acids, and developing scalable genome engineering technologies. Continued innovation in these areas will further strengthen the potential of GROs as versatile platforms for creating next-generation proteins and biomaterials.

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AUTHOR CONTRIBUTIONS

J.R and S.R contributed equally to the preparation and writing of the original draft. S.R led the conceptualization and manuscript drafting. J.R and S.R. prepared Figures; S.V.R and S.N provided critical review of original draft, P.M.S, R.R, M.K, N.S involved in editing of the manuscript. All authors have reviewed and approved the submitted version of the manuscript.

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