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Synthetic biology encompasses metagenomics, ecosystems, and biodiversity sustainability within its scope

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We envision the convergence of synthetic biology (SynBio) and metagenomics as a significant development for the engineering of complex biological systems. The entire biosphere with its diverse life forms can also be considered as a reservoir for evolutionary innovations and a source of modules for SynBio. Metagenomics, which is a large part of biodiversity, should be considered as an important source of modules. The abstraction hierarchy of amalgamating SynBio and metagenomics ("synthetic metagenomics") entails the standardized integration of parts, devices, circuits, and modules into functional chassis. These principles transcend the boundaries of single cell design and apply to the engineering of biodiversity sustainability in multicellular entities, their interconnections, and their dynamics in communities and whole ecosystems. Examples include applications in environmental sustainability, such as analysis of antimicrobial resistance in waste management, bioremediation of oil spills, and degradation of plastics. Future research and experimental interventions will ultimately provide a strong link between bioengineering, metagenomics, microbial consortia, ecosystems, and biodiversity sustainability under the umbrella of synthetic biology.

KEYWORDS

circuits, chassis, devices, biodiversity, bioengineering, metagenomics, modules

1 Introduction

Synthetic biology (SynBio) aims to use a combination of natural or artificial life components and apply multidisciplinary engineering principles to biotechnology (Acevedo-Rocha and Budisa, 2016). SynBio has essentially "borrowed" the concepts from software or electrical engineering for the design of basic functional units (usually bottom-up) that could be called modules (Endy, 2005). Thus, SynBio is a technoscience based on the fundamental idea that complexity can be created through the integration, combination, or arrangement of modules as interchangeable systems (de Lorenzo, 2011). Indeed, the inherent "constructability" of living organisms enables the division of complex systems into smaller, independent subsystems that can be used for different applications (Schmidt et al., 2018). To achieve this, standardization, abstraction, and modularity are required, with modules referring to functionally independent units with minimal interactions (orthogonality) (Costello and Badran, 2021). To design complicated biological systems, a common understanding of the functionality of these modular parts is critical. In addition, these parts should be, standardized, well characterized and easily accessible (e.g., BioBrick assembly of the MIT Registry of Standard Biological Parts (Sleight

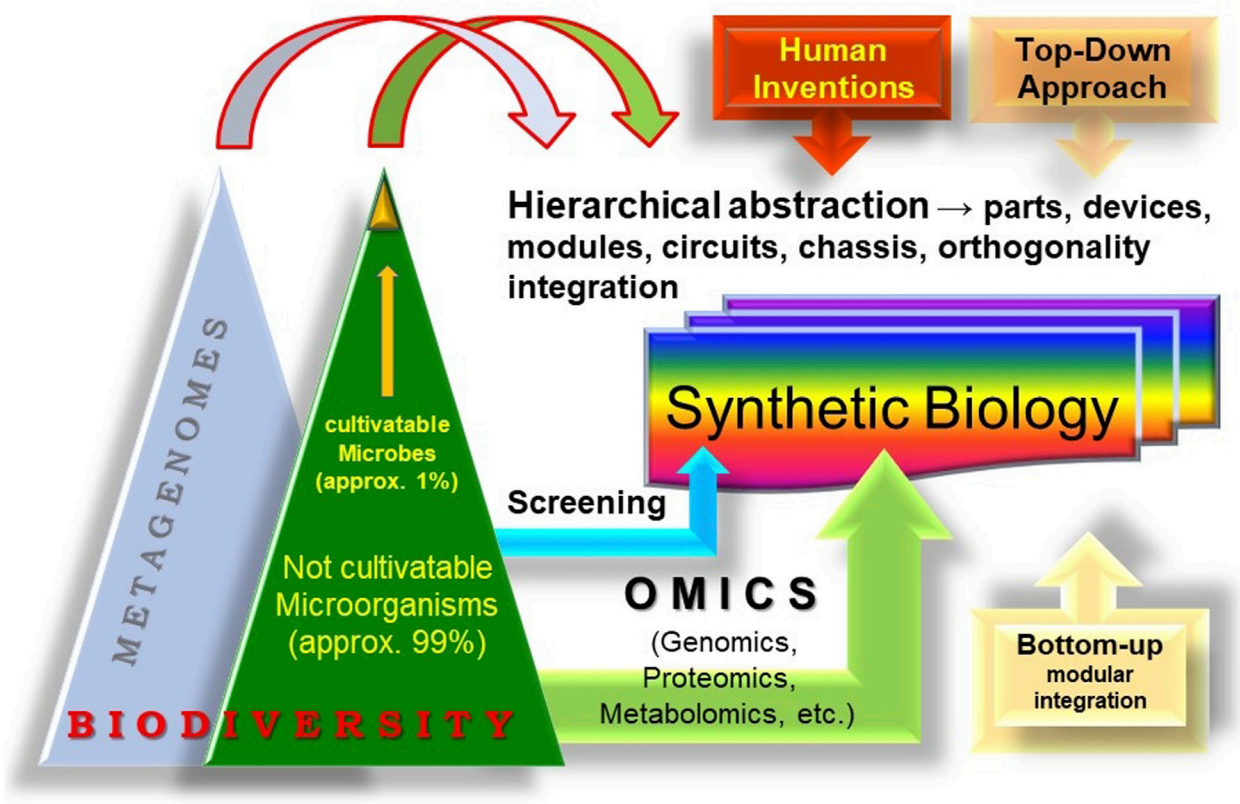

FIGURE 1
The concept of "synthetic metagenomics." The entirety of the biosphere, rich with its diverse life forms can also be considered as a reservoir for evolutionary innovations and a source of modules for Synthetic Biology. Metagenomics, which undoubtedly constitutes a large part of biodiversity, should be considered as an important wellspring of modules. The establishment of standardized procedures can leverage the bioengineering of these modules in specific contexts to create new biobased technologies and economies and even synthetic cells and communities.

et al., 2010b)). For this reason, SynBio is often assumed to be the computational analysis and modelling of complex biological systems (Chandran et al., 2008; Zomorrodi and Segrè, 2016). But it is more than mathematical modelling and tinkering by engineers of different domains.

Modularity and orthogonality are the basis for a robust and scalable circuit design inspired by natural gene circuits. These gene circuits rely on regulatory factors for cellular functions, using isolated and interacting components to achieve specific responses. Natural circuits consist of distinct functional modules, enabling adaptability to changing environments. Since the advent of recombinant DNA technology, researchers have extracted and modified elements from these circuits to create increasingly complex designs. As a result, versatile tools and components are available for the engineering of gene circuits. This journey has been accompanied by the expansion of synthetic biology tools from basic gene expression control and pathway adaptation (e.g., promoters, transcriptional activators/repressors, DNA/RNA/protein control and modification, molecular memory, etc.) to advanced genome editing control (e.g., CRISPR/Cas9, transcriptional regulation), multicellular pattern formations, genetic code expansion and engineering, and the recent innovation of synthetic cells and xenobiology [reviewed in e.g. (Bradley et al., 2016; Budisa et al., 2020)]. The latter is an excellent example of the process of assimilation of non-native substrates, also referred to in the literature as synthetic heterotrophy (Sullivan et al., 2023), which is relevant not only to the design of synthetic life but also to cell regulation, as it is a technology that diverts synthetic metabolism to optimize biomass formation.

A "device" in SynBio refers to a combination of some biological parts that work together to produce a particular useful function or behavior (Müller and Arndt, 2012). These devices can be further integrated or scaled to form more complicated structures. For example, a genetically engineered bacterium created for a specific purpose, such as the degradation of plastic polymers into monomers, will have both the ability to convert monomers into biomass through anabolic processes and catabolically "burn" the hydrolysis products of the degraded polymer. In this scenario, the polymer-degrading "device" (e.g., the catalytic enzyme cascade) and the biomass-producing "circuit" (e.g., synthetic metabolism) are functionally independent, but interact at the interface level in a controlled and predictable manner. These hierarchical levels of complexity can be described as "systems" where the combined behavior of different devices and circuits results in holistic behavior phenomena (Delgado and Porcar, 2013). Finally, the term "chassis" refers to living host that nurtures and sustains the genetic elements, systems, and processes (as described above) by providing the resources necessary for their proper functioning (Danchin, 2012).

In this context, the integration of metagenomics into SynBio promotes the pursuit of advanced modular design that leverages host cells for tailored bioactivities by tapping into the genetic and biochemical diversity in the genomes of microorganisms from environmental samples or through metagenomics (Figure 1). We anticipate that this integration will significantly boost the whole SynBio field, as its basic principles (modular integration, standardization, abstraction, and orthogonality) are applicable to the engineering of biodiversity sustainability both in single cells, and in multicellular entities, their interconnections and dynamics in communities and whole ecosystems.

2 Topics relevant for the subject

2.1 Metagenomics

Metagenomics is a field of research that focuses on the study of genetic material obtained directly from environmental samples such as soil, water, or even the human gut (Yen and Johnson, 2021). Unlike traditional genomics, which focuses on the DNA of individual organisms, metagenomics examines the collective genetic material of all microorganisms present in a given sample (New and Brito, 2020). The core idea of metagenomics is to analyze the genetic diversity and functional potential of entire microbial communities without the need to isolate and culture individual organisms (Berini et al., 2017). This approach allows us to gain insights into the composition, structure, and functional capabilities of complex microbial ecosystems that are difficult to study using conventional molecular biological methods (Garrido-Cardenas and Manzano-Agugliaro, 2017).

Metagenomics involves sequencing and analyzing the DNA or RNA present in a sample from a particular ecological niche (Marco, 2008). Because this material is normally fragmented and piecemeal, advanced sequencing technologies are used to retrieve genetic information. By analyzing the sequences, it is possible to identify the species of microorganisms present, their relative abundance and the functional genes they carry (Kayani et al., 2021). This information can provide valuable insights into ecosystem dynamics, microbial interactions, and potential applications in biotechnology and environmental research as elaborated in our study. In principle, metagenomics allows us to explore the genetic composition of entire microbial communities in their natural habitats and thus discover the hidden biodiversity and functional potential of these ecosystems (Bouhajja et al., 2016). Here, we describe possible pathways to "synthetic metagenomics" (Figure 1) by merging SynBio with classical metagenomics.

In this context, metagenomics is evolving into a technology that complements gene and protein engineering methods and applies SynBio's system design concepts (see above). Metagenomics exploits the wealth of genetic and biochemical diversity present in the genomes of microorganisms in environmental samples (Figure 1) and offers a range of new technologies for screening novel catalytic activities from environmental samples with potential biotechnological applications (Guazzaroni et al., 2015) or even diagnostics (Greninger, 2018). For example, metagenomic analyses of marine ecosystems have begun to reveal the extent of novel compounds encoded in the vast bacterial richness and diversity of the marine ecosystem (Tseng and Tang, 2014). A combination of unique physicochemical properties and spatial niche-specific substrates in wide-spread and extreme habitats underscores the potential of the marine environment to deliver functionally novel biocatalytic activities (Parages et al., 2016).

In recent years, metagenomics has expanded our understanding of how the collective functions of microorganisms emerge from the complex web of interactions between their genetic and metabolic potentials and the environment to enable useful applications in terms of environmental sustainability (Sharma et al., 2022). A particular focus of metagenomics is the study of the interactions of environmental pollutants (xenobiotics) of anthropogenic origin (e.g., plastics, pharmaceuticals, pesticides, cosmetics, flavorings, fragrances, food additives, industrial chemicals, and plastics) with the local environment and their global impacts (Danso et al., 2019). Metagenomic analyses have expanded our understanding of ecosystems and biodiversity in several fields such as antimicrobial resistance in waste management systems, bioremediation of oil spills, and biodegradation of plastics.

Therefore, synthetic metagenomics can be seen as an emerging field of research in which metagenomes are studied functionally following the key postulates of SynBio (see above). This approach substantially expands the set of identified biological activities and building blocks. For example, functional metagenomic of metabolic pathways in microbial communities has led to the discovery of novel bioactivities such as amidases, NF-κB modulators, naphthalene degrading enzymes, cellulases, lipases, and transporters (Robinson et al., 2021). Using these genetic devices and circuits as templates along with their further functional integration, improvements can be made by altering or redesigning metabolic pathways in selected microbial chassis to improve synthesis and yield of high-value products (van der Helm et al., 2018).

2.2 Antimicrobial resistance

The emergence and the spread of antimicrobial resistance in animal husbandry is a growing global concern (Karbalaei-Heidari and Budisa, 2020). Metagenomics has proven to be a powerful tool for analyzing the evolution of antimicrobial resistance genes (ARGs) and mobile genetic elements (MGEs) in microbiome of animals. Large quantities of veterinary pharmaceuticals and drugs are used not only to treat infectious diseases, but also as prophylactic therapies and feed enhancers, potentially leading to the selection of ARGs, MGEs, and ultimately, antimicrobial resistance bacteria (ARBs). For instance, the study of the "Resistome" (complete set of ARGs) in ruminal microbiomes has shown the presence of ARGs encoding resistance to tetracyclines, one of the most used antibiotics in farms, in the genomes of common ruminal species (Sabino et al., 2019). Not surprisingly, animal manures are significant reservoir of ARGs and MGEs and can promote the development of ARBs, which are often spread in agricultural fields when manure is applied as fertilizer (Buta-Hubeny et al., 2022).

In this context, metagenomics and microbial diversity analyses provide a valuable framework for studying the impact of different manure treatments on resistomes and mobilomes (complete set of

MGEs). Through these technologies, we are gaining a better understanding of the microbial species that harbor antimicrobial resistant elements and the dynamics that drive changes in ARGs and MGEs. For example, metagenomic analyses have shown that microbial successions during the mesophilic and thermophilic stages of manure composting or the establishment of anaerobic consortia during anaerobic digestion (AD), can reduce the abundance and diversity of ARGs and MGEs in animal manure (Cheng, 2017; Flores-Orozco et al., 2023; Flores-Orozco et al., 2023). The knowledge gained from these applications is crucial for addressing the serious health problem that antimicrobial resistance poses and for developing strategies to minimize the risk of its spread in agriculture.

2.3 Bioremediation of oil spills

Advances in microbial genetics and "omics" techniques have facilitated the analysis of microbial communities beyond the limited reach of conventional culturing methods, which generally focus on the growth and activity of single organisms under optimal laboratory conditions. Metagenomics has expanded scientific knowledge of complex microbial communities, enabling analysis of interactions among microbes in the community, as well as co-metabolism, enzyme activity, and biodegradation of oil in the natural environment. "Omics" tools, such as metagenomics and meta-transcriptomics give researchers and spill responders the ability to assess the biodegradation capacity of the native microbial community at a spill site and provide them with further information on whether and to what extent further action is needed (Czaplicki and Gunsch, 2016; Ghosal et al., 2016; Techtmann and Hazen, 2016; Mukherjee et al., 2017).

Oil is a naturally occurring, non-renewable energy source that can cause severe detrimental environmental damage if released in large quantities, such as in an accidental spill. Crude oil is a mixture of thousands of compounds, of which polycyclic aromatic compounds (PACs) are of particular concern due to their acute and chronic toxicity, mutagenicity, and carcinogenicity to interacting organisms (Cao et al., 2009; Dupuis and Ucan-Marin, 2015). PACs consist of two or more fused benzene rings and vary in structure, complexity, and recalcitrance (Haritash and Kaushik, 2009; Lee, 2015). The larger the compound, the more persistent it is likely to be in the environment (Rosenberg and Ron, 1996). To limit the impact of oil on habitats, biota, and nearby communities, mechanical and chemical measures are commonly used in oil spills to facilitate removal or degradation of the oil. However, some of these conventional methods are invasive and cause damage to sensitive habitats and leave oil behind (Zhu et al., 2001). Increasing research and attention has been given to non-invasive alternatives in which native microorganisms play a role in removing oil compounds from the environment after an oil spill (Ghosal, Ghosh, Dutta and Ahn, 2016).

The potential of bioremediation to fill the sustainability gap is substantial when guided by the right insights. The refinement of bioremediation practices can be achieved through the integration of novel "OMICS" methodologies aimed at deciphering the microbiome associated with hydrocarbon plastics. To attain this objective, an exhaustive understanding of bacterial and fungal degradation pathways, their intricate interplays within microbiomes, and their interactions with the soil matrix is imperative. Leveraging "OMICS" techniques facilitates the investigation of both cultivable and non-cultivable soil microbial communities engaged in degradation processes, offering tools to pinpoint pivotal organisms responsible for degrading soil pollutants and reinstating soil vitality and resilience. To do this, we need to thoroughly investigate microbial communities, their degradation pathways, and their complex interactions. Numerous studies provided detailed explanations of genomic and metagenomic methods, as well as the tools necessary to decipher the information obtained. These methods are applicable in two ways: first, to refine bio-based strategies for effectively treating soils contaminated by petroleum hydrocarbons, and second, to facilitate the smooth up-scaling of these technologies to industrial scales (Chicca et al., 2022; Chicca et al., 2022; Becarelli et al., 2023).

2.4 Biodegradation of plastics

Petroleum-based synthetic plastics such as polyethylene (PE), polypropylene (PP), polyvinylchloride (PVC), polyethylene terephthalate (PET), and polystyrene (PS) are the indispensable components of modern industry and daily life but have caused significant environmental problems world-wide due to their extreme recalcitrance to biodegradation in the environments (Krueger et al., 2015; MacLeod et al., 2021). Since current management strategies such as landfills, incineration, and mechanical recycling, are not sufficient to cope with the increasing amount of plastic waste (Drzyzga and Prieto, 2019; Lim and San Thian, 2022; Ackerman and Levin, 2023), biodegradation of these plastics and biotechnological recycling have emerged as an alternative and become one of the research areas actively studied worldwide. Several studies also showed that certain microbial groups have been enriched in plastic films or microplastics in terrestrial environments. Chung et al. (2022) compared the microbial communities on the plastic waste films, which were mostly made of PE and had been buried in the four landfill sites for more than 20 years, with those in the nearby soils assuming that specific and plastic-degrading microorganisms were enriched on the WPFs. The Fourier-transform infrared (FTIR) spectroscopy showed that the WPFs were oxidized, though not as much as when exposed to UV-light and oxygen.

Microbial communities were primarily characterized by their geographical origins, but communities on WPFs generally had lower species richness and diversity than those in nearby soils and exhibited community structures that differed from those in nearby soils. The species taxonomically close to *Bradyrhizobiaceae*, *Pseudarthrobacter* (formerly known as *Arthrobacter*), and *Bacillus* in the bacterial communities and the one close to *Mortierella* in the fungal communities were enriched on the WPFs, suggesting that they might be involved in the degradation of the plastic waste films. The network analysis also indicated that the species related to *Mortierella* was one of the keystone species on the WPFs. Several studies reported that *Arthrobacter* and *Mortierella* were also enriched on plastics in

soils (Gao et al., 2021; Wright et al., 2021; Yi et al., 2021) or had the ability to degrade plastics (Albertsson et al., 1998; Han et al., 2020). For example, metagenomic analyses have shown that specific and common microbial groups adapt to plastics in terrestrial and marine environments. While there is not yet direct evidence that these microbes are directly responsible for degrading plastics is not yet available, SynBio technologies may soon confirm this hypothesis.

3 Discussion and outlook

The combination of smaller subsystems that arise independently and are later integrated into a whole with new functions is precisely how ecosystems evolved their metagenomics and enormous biodiversity. Therefore, the fundamental principles of SynBio are entirely compatible with our perceptions of ecosystems and their sustainable development. In this context, metagenomics is the study of the structure and function of entire nucleotide sequences isolated and analyzed from all organisms (typically bacteria and fungi) in a bulk sample, based on the direct extraction and cloning of DNA from their natural environment. Metagenomics is often used to study a specific community of microorganisms, such as those residing on human skin, in the soil, or in a water sample. The difference between genomics and metagenomics is in the nature of the sample. Genomics explores examines only the complete genetic information of a single organism only, whereas metagenomics explores a mixture of DNA from multiple organisms and entities within a microbial community, and which may also include viruses, viroids, and free DNA.

Metagenomics samples undoubtedly hold great potential for the introduction of new technologies, e.g., by screening for novel catalytic activities in these samples. This should primarily lead to the development of new-to-nature (i.e., artificial) functions and systems, such as bacterial photography, tumor targeting bacteria, tunable oscillators, sensors, and counters (Sleight et al., 2010a). The integration of novel catalytic activities into controlled synthetic heterotrophic metabolism (Bayer et al., 2009) should facilitate genetic re-engineering of microorganisms e.g., combining metabolic engineering and protein engineering (Völler and Budisa, 2017) for practical applications such as degradation of plastics or remediation of xenobiotics. The production of valuable chemicals or raw materials such as detergents, antimalarials, biofuels from plant biomass, valuable components produced by adaptive evolution in the laboratory, or even a synthetic chromosome transplanted into a host are just a few examples that are well documented in the current literature (Wang et al., 2019).

Metagenomic samples often contain unique genetically encoded information derived from different spatial habitats, e.g., extreme environments (hot, cold, acidic, basic, salty), and often offer fascinating new biological activities. However, a key challenge is to find a compatible host or chassis capable of functionally expressing metagenomic DNA. Species-specific differences in metabolic profiles, regulatory networks, transcription, translation, and post-translation mechanisms complicate this endeavor (Amarelle et al., 2023). Therefore, the introduction of standardized hosts (chassis) and SynBio principles is essential to effectively exploit the biological innovations of metagenomics. These adaptable chassis, which include mesophilic, psychrophilic, thermophilic, halophilic, and other species, should be standardized platforms designed to house built-in metagenomic libraries. For example, live bacterial therapeutics could use microbial hosts (e.g., *Escherichia coli*-based chassis) to ensure resilient colonization and drug delivery in challenging luminal environments (Russell et al., 2022). Other approaches include finding standardized chassis for genomic clusters encoding various natural products (Beites and Mendes, 2015; Liu et al., 2022), or synthetic metagenomics in yeast—a concept has the potential to reproduce the metabolic network of an entire ecosystem in a single *Saccharomyces cerevisiae* cell (Belda et al., 2021).

Therefore, the integration of metagenomics, ecosystems, and biodiversity sustainability in SynBio is a compelling vision (Figure 1). The conceptual framework of SynBio, which recognizes the hierarchical structure of genes to genomes and extends to higher levels of complexity (Endy, 2005), shares many commonalities with the above topics. In addition, Systems Biology and mathematical modeling will serve as foundational elements in considering the modular redesign of individual life forms and their communities (Porcar et al., 2013). This will allow us to conceptualize and even design complex communities and environments such as the human gut, e.g., through the directed evolution of microbial species, subpopulations, and entire populations as components of a given ecosystem or microenvironment (Bacchus and Fussenegger, 2013). Not surprisingly, Synthetic Microbial Consortia have gained prominence in SynBio almost a decade ago (Shong et al., 2012). Future studies and experimental interventions, including bioremediation, will explore the intricacies of microbiomes, diverse ecological niches, and environments that are highly contaminated by pollutants such as heavy metals, plastics, oil spills, and xenobiotics.

This comprehensive approach will ultimately provide a strong link between bioengineering, metagenomics, microbial consortia, ecosystems, and biodiversity sustainability in Synthetic Biology. The ultimate goal of these efforts would be to create a portfolio of technologies for a long-term, sustainable solution to critical global problems such as marine pollution reduction, climate change mitigation, food security, and biodiversity conservation.

Author contributions

DL: Conceptualization, Funding acquisition, Investigation, Project administration, Resources, Validation, Visualization, Writing–original draft, Writing–review and editing. NB: Conceptualization, Writing–review and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Publisher's note

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