The Authenticity of Synthetics

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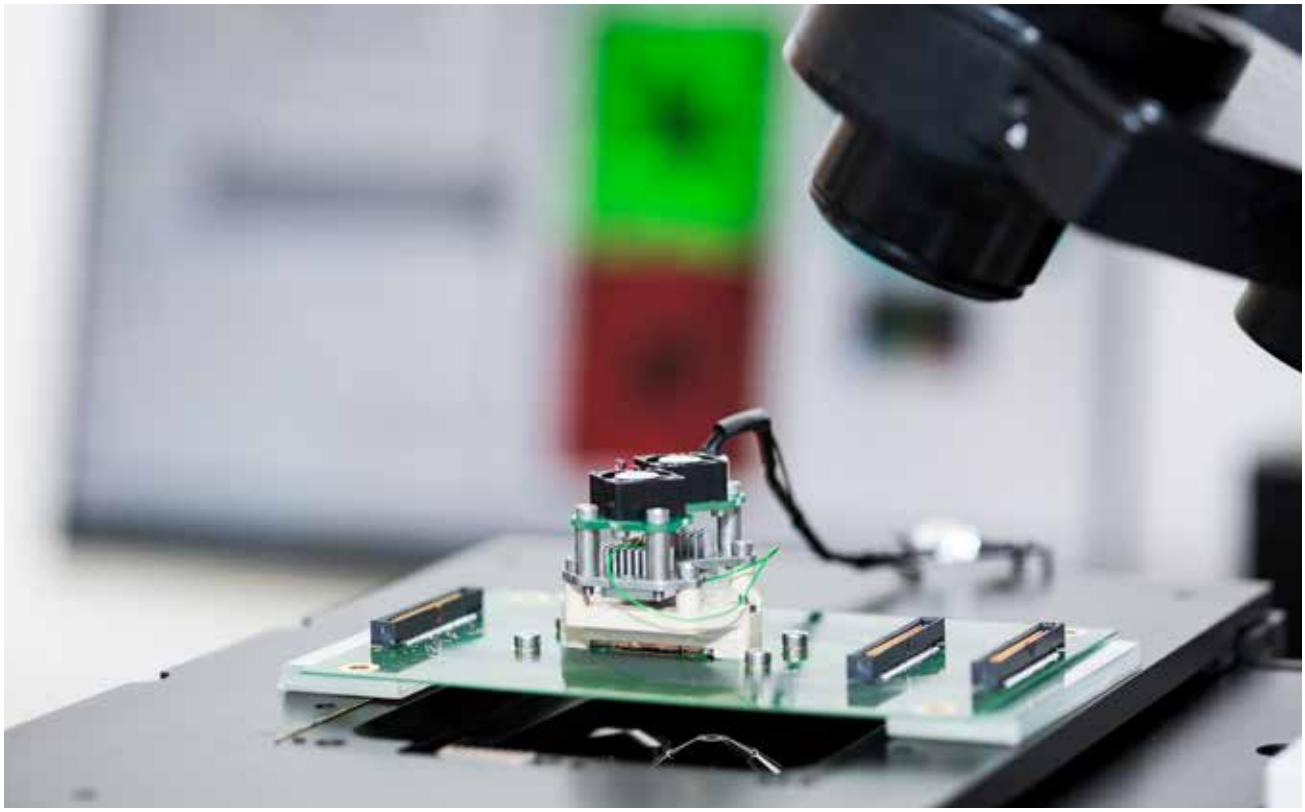
Synthetic biology constructs novel artificial biological pathways and organisms or redesigns existing natural biological systems. This is leading to great expectations of noteworthy and widespread impact in the drug discovery and development field

Dr Tim Brears at Evonetix

Synthetic biology is a newly coined term for the design and construction of novel artificial biological pathways, organisms, or devices or the redesign of existing natural biological systems (1). Generally, it assumes an element of scale and the application of engineering-like techniques to solve biological problems through a rational process of 'biodesign'. The field of synthetic biology brings together skills from biology and chemistry to computing, bioinformatics, and engineering. This emerging field is greatly anticipated to deliver significant and broad impact across a range of potential applications, among them the drug discovery and development field. However, to achieve its full potential, synthetic biology will require the continued development and convergence of its underlying skills and disciplines to enable the rational and predictable design of biological systems. As DNA is a fundamental building block of synthetic biology, the key to its success will be the ability to synthesise DNA at scale and with high accuracy.



The discovery and development of novel pharmaceuticals has undergone various changes, from the use of natural products to the development of synthetic chemistry as a source of new ingredients and products, the use of antibodies and other biologics, to the emerging potential of gene therapy and personalised medicine. With the advent of synthetic biology, the ability to explore, select, and optimise the biology around us is greatly enhanced, and with this comes the opportunity to streamline the discovery process and develop novel solutions based directly on the underlying biology.

Screening for New APIs

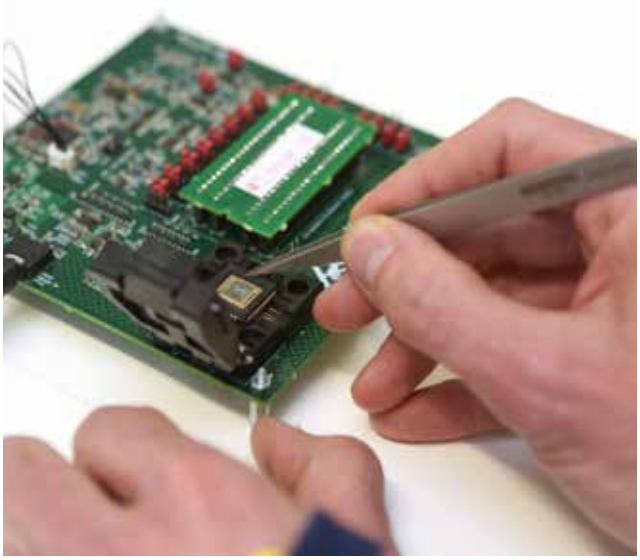
Historically, drug discovery has been led by the screening of small molecule chemical libraries against targets of therapeutic interest. The targets themselves often require heterologous expression, and the tools of molecular biology can be applied at various stages of the assay generation process to, for example, improve the expression of screening targets and reporters, thus improving the throughput and read-out of these systems.

For many years, pharma drug discovery has relied heavily on the libraries of (typically) small molecules used in the screening process. More recently, synthetic biology also offers the possibility of generating large libraries of screening compounds that are genetically encoded or produced enzymatically. While chemically synthesising cyclic peptides is possible, genetically encoded libraries have several advantages as they are more readily accessible and allow straightforward hit deconvolution (2). This class of compound includes vancomycin (the well-known antibiotic), cyclosporine (immunosuppressant), and actinomycin (anti-cancer drug).

Polyketides, such as anti-cancer agents (eg, epothilone), antibiotics (eg, erythromycin), and immunosuppressants (eg, FK-506) represent an important class of natural products and are built in a modular fashion using polyketide synthases. The genes encoding polyketide synthases can be recombined to create libraries of polyketides that can then be screened for novel biological activities.

Protein Engineering for the Design of Novel Therapeutics

The era of biologics as a class of drug produced through recombinant DNA technology started in 1982 with the market introduction of Humulin®, human insulin made using



recombinant DNA. Almost 40 years later, the tools of synthetic biology provide the ability to engineer novel proteins for use as therapeutics on a scale not previously possible. By rationally designing many variants, guided by protein structural expertise, generating therapeutic proteins with desirable properties in a process akin to directed evolution is possible.

One such example is the generation of protein variants of phenylalanine hydroxylase (PAH) with gastrointestinal (GI) stability, which survive degradation in the GI tract to treat phenylketonuria, a rare, autosomal recessive condition characterised by high levels of phenylalanine due to a mutation in the gene encoding PAH (3). PAH is a hepatic enzyme that converts phenylalanine into tyrosine and is subject to a proteolytic attack if administered orally. Creating a GI-stable protein variant of PAH allows the oral administration of a therapeutic enzyme to compensate for the absence of the natural enzyme by hydroxylating phenylalanine, reducing the risk of neurological complications associated with high levels of phenylalanine accumulation in the patient.

A further example is provided by the engineering of an entirely novel protein that degrades gluten to treat coeliac disease (4). Using computational design, a recombinant enzyme was developed to target the major immune-reactive protein component of gluten and gliadin under acidic stomach conditions. Gliadin is enriched in the amino acids proline and glutamine, which are difficult to digest and trigger the inflammatory immune response, leading to coeliac disease. Novel enzymes that have high specificity for this protein can significantly reduce the risk of intestinal damage and improve the lives of patients.

Biosynthesis of Pharmaceuticals

Although recombinant DNA technology has long been used to synthesise biologically active compounds in novel hosts, the advances and potential scale presented by synthetic biology offer new opportunities in this exciting arena.

More than 50,000 terpenoids exist which are important secondary metabolites. Synthetic biology offers a novel solution to increase the speed of, and reduce the costs associated with, producing one such terpenoid, the antimalarial drug artemisinin, which is costly and onerous to cultivate from its natural source. To overcome these barriers, scientists engineered genetically modified strains of *Saccharomyces cerevisiae* to produce artemisinic acid, a precursor of artemisinin, making this lifesaving drug faster and cheaper to manufacture (5). This excellent example provides an indication of the power of synthetic biology and could enable significantly more widespread use of artemisinin-based combination therapies for the treatment of malaria as recommended by the WHO (6).

However, the same level of success has not been achieved for the terpenoid paclitaxel, a well-known antineoplastic agent. Paclitaxel is used in chemotherapy to treat a variety of cancers and is produced by the bark of the Pacific yew tree (*Taxus brevifolia*). It is currently sourced either using a semisynthetic method from *Taxus baccata* (European yew) or from a plant cell fermentation technology in which a specific Taxus cell line is fermented (7). Paclitaxel is synthesised in approximately 19 enzymatic steps from geranylgeranyl diphosphate, and, although many efforts have been made to achieve total synthesis in an appropriate host organism, this has not proven commercially viable due to its complexity.

Synthetic biology clearly has great potential for the heterologous production of natural products. Starting with pathways known in specific organisms, re-cloning, codon optimisation (for the new host), and generation of gene variants to optimise activity can become a reality with access to synthetic DNA.

Where Does the Future Lie?

Beyond the examples discussed above, the impact of synthetic biology on drug discovery will be broad, including the development of novel vaccines (eg, antigen genes or nucleic acid vaccines), gene therapy (with its requirement for synthetic DNA), CRISPR-Cas9, and other cellular engineering techniques.

However, the opportunity goes further than this. The expanding body of sequence information will uncover a universe of hitherto unknown opportunities. For example, the wealth of natural products found in GI-inhabiting microbes (including many new antibiotics) must surely represent a huge opportunity, realisable as more effective skills to identify, analyse, and optimise the expression of the underlying genes in preferred hosts are developed.

Going beyond its well-understood biological function, DNA as a structural molecule offers opportunities in human healthcare that could not have been anticipated in the relatively recent past. Using 'scaffolded DNA origami', making structures that are stable in physiological environments and that could be used to deliver therapeutic molecules is possible (8).

Overcoming the Challenges with DNA Synthesis

Synthetic biology is enabled by the vast amounts of DNA sequence information available from a huge array of organisms and the availability of genomic and metagenomic tools to understand the potential functionality of such information.

Clearly, synthetic biology has the potential to empower many aspects of drug discovery. However, the scale of this impact relies on the continued development of its underlying technologies, including bioinformatics, the identification of suitable host organisms and reliable methods of designing biosynthetic pathways, and DNA synthesis.

Further development of synthetic DNA synthesis is crucial for the achievement of many of the opportunities in synthetic biology. Whereas DNA sequencing underwent a revolution in the mid-2000s, resulting in massive parallelisation and reduction in cost, a comparable breakthrough in DNA synthesis is now required.

While companies have already made solid progress in upscaling DNA synthesis, challenges remain in achieving further scale, greater accuracy, and reduced cost. Overcoming some of the challenges to allow robust, scalable, and cost-effective synthesis of high-fidelity DNA will enable DNA synthesis to enjoy a sea change similar to sequencing in the mid-2000s.

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About the author

Dr Tim Brears is CEO at Evonetix, where he leads the company in developing a bench-top DNA writer to transition DNA synthesis from a service industry to one where scientists can make genes at will. He holds a PhD from the University of Cambridge, UK, and has an MBA from Duke University's Fuqua School of Business, US.
Email: tim.brears@evonetix.com