

# Drug

TARGET REVIEW

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Volume 9 · Issue 03 · Autumn 2022

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to deliver therapeutics

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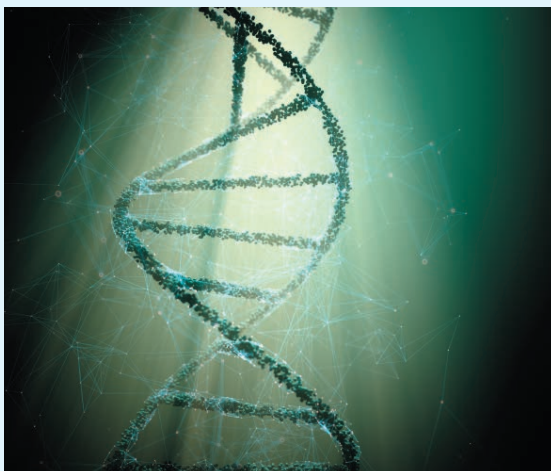
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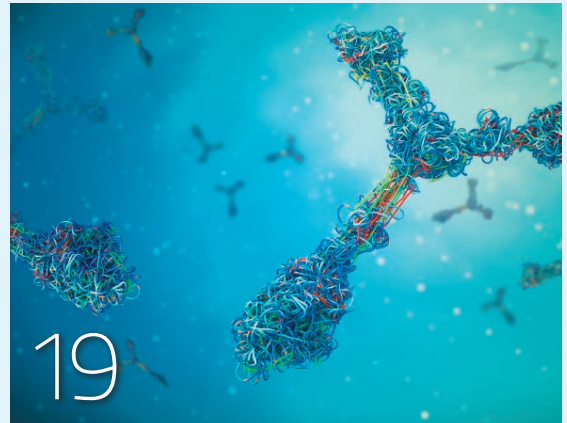
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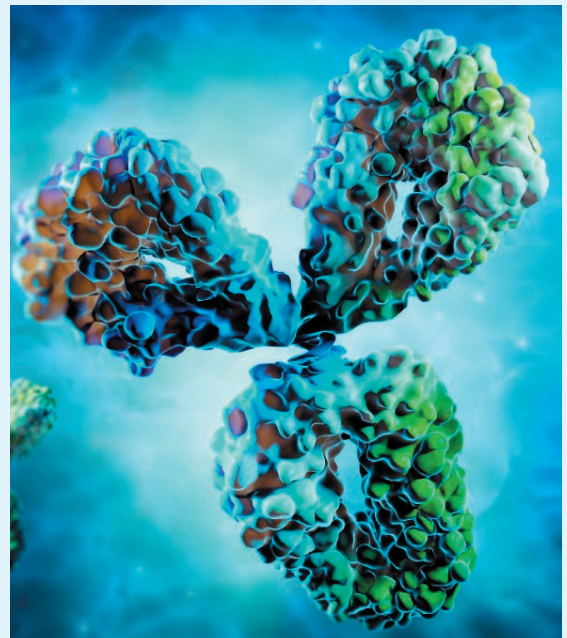
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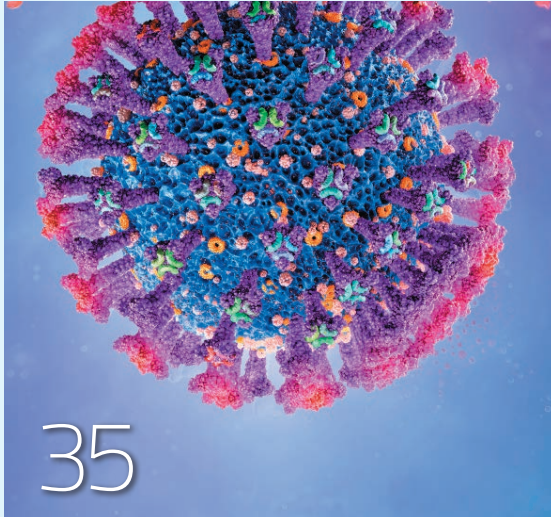
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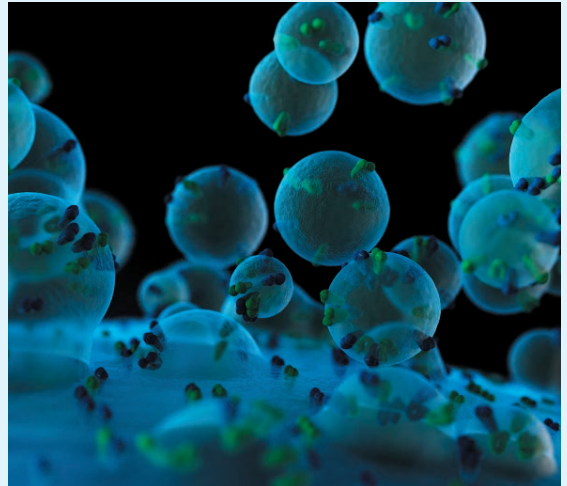
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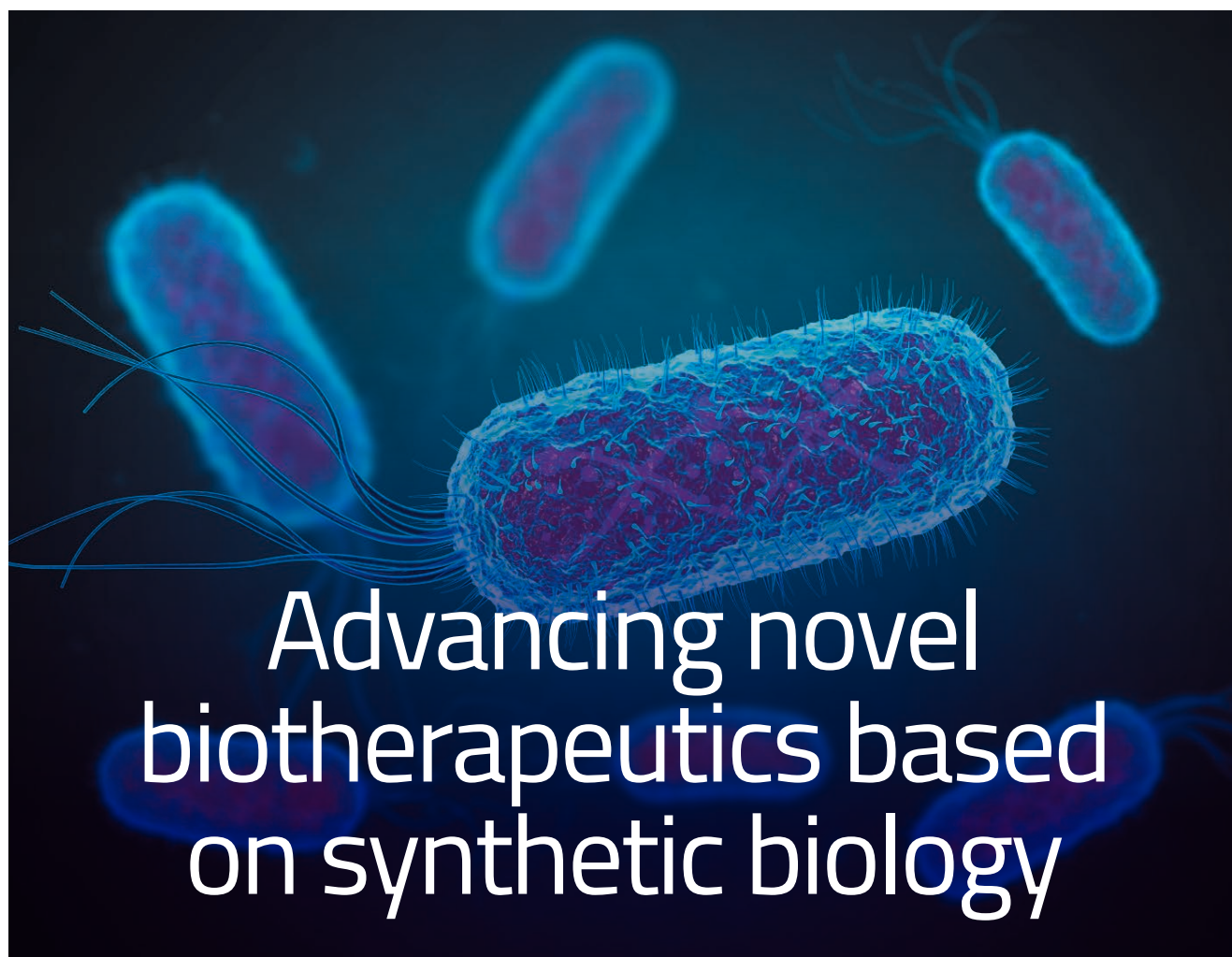
# Synthetic Biology

Synthetic biology has opened up the possibility of entirely new classes of medicines. Here, Dr Caroline Kurtz, Synlogic, discusses synthetic biotics and their potential to be key in the treatment of rare diseases, metabolic conditions, autoimmune diseases and more. In another piece, Dr Dan Mandell, GRO Biosciences, describes how the field of synthetic biology has developed over the past 20 years and outlines where it is likely to go in the future in regards to therapeutic applications.

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# Advancing novel biotherapeutics based on synthetic biology

Researchers at Synlogic are clearing the path for a new class of medicine – biotherapeutics based on synthetic biology, called synthetic biotics, which are created by programming or engineering bacteria to metabolise or secrete well-validated targets of disease pathophysiology. In this article, Dr Caroline Kurtz, Chief Development Officer at Synlogic, discusses how synthetic biotics work and their potential applications in the treatment of a broad range of conditions including rare diseases, metabolic conditions, autoimmune diseases and inflammatory diseases.

**T**HE CONCEPT of synthetic biology emerged as early as the 1960s when researchers started to advance precision genetic engineering techniques and apply them to drug development. Simultaneously, advances in molecular biology, DNA sequencing, understanding of gene expression and regulation of cellular function collectively made it possible to engineer bacteria and develop new drugs ready to offer therapeutic benefit to patients living with many serious diseases. After years of continued research and innovation, synthetic biology is now a rapidly growing area of biomedical discovery and drug development.

Founded in 2014 by Jim Collins and Tim Lu, both professors from Massachusetts Institute of Technology (MIT), US and world-renowned experts in synthetic biology, Synlogic was one of the first companies established with the goal of developing biotherapeutics based on synthetic biology. Since then, the company has made rapid progress in advancing promising research, with six Investigational New Drug (IND) applications filed with the US Food and Drug Administration (FDA), four investigational therapies in development and treatment experience in more than 350 patients in clinical programmes. Synlogic leverages a range of synthetic biology tools to

design and develop therapeutics based on genetically engineered bacterial microbes, with a current focus on treatments for metabolic and immunological diseases including phenylketonuria (PKU), homocystinuria (HCU) and enteric hyperoxaluria (EH).

## How synthetic biotics work

While synthetic biology has applications in a wide range of industries, in drug development, this therapeutic approach uses genetic engineering to programme cells and bacteria to perform a specific function or deliver a therapeutic agent able to activate, inhibit or modulate a function in the body to treat disease.

At Synlogic, researchers use a well-characterised probiotic bacterium called *E. coli* Nissle 1917 as the microbial "starter strain" or chassis. *E. coli* Nissle has an advantageous safety profile validated by more than 100 years of clinical research and therapeutic use in humans. In targeting metabolic and immunological diseases, the Synlogic team engineers *E. coli* Nissle to play a precise role in the gastrointestinal (GI) tract, focusing on the degradation and production of metabolites that are relevant to disease.

Leveraging the advantages of the *E. coli* Nissle bacterium is only part of the equation that leads to the production of synthetic biotics. Synlogic researchers also apply their expertise in disease physiology, molecular biology and microbial engineering and combine these skills with the company's library of proprietary tools and modular components including metabolic gene circuitry and regulatory genetic switches. Together, these components allow researchers to design synthetic biotics based on a series of complex steps and then deliver them to patients.

#### Assessing the drivers of disease

First, we conduct a comprehensive assessment of available biological data and scientific literature to understand the underlying mechanisms or metabolic dysfunctions that cause different diseases

and then confirm the potential molecular targets. Our goal is to identify diseases in which we could have a meaningful impact and can determine the efficacy and safety of drugs based on the demonstrated mechanism of action of our proprietary synthetic biology platform. This approach led us to establish our initial focus on treatment of rare metabolic diseases including PKU and enteric hyperoxaluria. We used predictive modelling and bioinformatics to better understand the levels of target metabolites involved in these diseases and whether they are in an optimal range that would make synthetic biotics effective as a potential treatment option.

#### Designing and testing potential solutions

In the next step, we build synthetic biotic prototypes and test their ability to carry out specific functions that may treat a target disease, such as consuming toxins or regulating cellular function. We also investigate whether they meet pre-defined criteria in terms of their mechanisms of action in *in vitro* models. In these processes, we use DNA assembly to target candidate drug-induced pathways and mathematical models to predict a pathway's potential efficiency. We also use proprietary switches to permit control and modification of engineered pathways or circuits with our probiotic *E. coli* Nissle microbial chassis to carry a therapeutic.

#### Building and administering synthetic biotics

Once we identify, test and validate the optimal components needed for our synthetic biotics, we insert them into the genome of the microbe. Our proprietary integration systems can direct insertion of genetic circuits into the *E. coli* Nissle chassis while ensuring genetic stability. Through our unique manufacturing process that involves automated capabilities, single-use technologies and co-location of fermentation, downstream processing and lyophilisation, we can produce synthetic biotics with high drug viability, which is imperative to their proper function in disease targeting. Our synthetic biotics are formulated into a stable powder with the convenience of oral administration. This unique design process and therapeutic approach was first applied as a possible treatment for the rare genetic disease PKU.

#### PKU – a rare metabolic disease with a significant unmet need

In many cases, an important factor in metabolic disease pathophysiology is diet. Disease progression models and direct experience with disease management indicate that GI-based intervention to consume a dietary metabolite can potentially deliver efficacy benefit. Research at Synlogic has demonstrated >>

## EXPERT VIEW



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## DNA watermarks, an indelible tag for synthetic genes

DNA watermarking is a relatively new technology that embeds a unique DNA sequence into a synthetic gene. It has the potential for several applications including traceability, branding, ownership, warnings, dates and patent information. DNA watermarks are facilitated by *de novo* gene synthesis which enables the conversion of electronic sequence data into tangible biological molecules. It is easy to modify the sequence before synthesising the DNA to maximise expression or introduce other functional improvements.

Changing the sequence of a gene to add the watermark could interfere with function.

Using non-coding DNA addresses this challenge, but the watermark sequence could easily be altered or removed. The idea of watermarking is like labels on clothing. A shirt can be labelled with a tag in the neckline, where it has no other function to the shirt. This tag can easily be removed without destroying the shirt's function. Alternatively, the shirt can be labelled by weaving the logo of the company into the fabric. Removing this tag will ultimately destroy the shirt. Similarly, watermarks can be added to the coding region of a gene using different codons for the same amino acid. Once the DNA watermark has been incorporated it can be read by sequencing the gene of interest and

decoded by anyone with knowledge of the key for deciphering the embedded information.

Thermo Fisher Scientific supports gene synthesis for many applications. Customers wishing to incorporate their own watermarks or other unique gene sequences can quickly obtain several variants to test whether candidate sequence alterations cause a functional change. In addition, the proprietary GeneArt™ GeneOptimizer™ algorithm can be used to improve protein expression while critical motifs, such as introduced watermark sequences or cloning sites, can easily be protected from sequence changes during the optimisation step.



that synthetic biotic therapies are able to consume GI-based metabolites and affect their levels systemically, including plasma phenylalanine (Phe) for PKU, plasma methionine and homocysteine for HCU and urinary oxalate for enteric hyperoxaluria (EH). These insights have positioned Synlogic to advance development programmes in multiple disease targets including PKU, HCU and EH.

PKU is a rare, genetic metabolic disease caused by defects in phenylalanine hydroxylase (PAH), an enzyme that metabolises Phe, an amino acid found in many foods including meat, vegetables, breads and cereals. Phe buildup is toxic and can lead to severe neurological damage and neurocognitive defects as well as increase the risk of intellectual disabilities, deficits in mental processing, social engagement and emotional problems. Lifelong metabolic control of Phe is the key to reducing this risk, with many patients attempting to adhere to a strict low-protein diet that can be extremely challenging. While there are some approved treatments for PKU, a large majority of patients are unresponsive and others decline or discontinue treatment due to safety risks.<sup>1</sup>

The mechanism of action of disease progression in PKU represents a highly compelling target for drug development based on synthetic biology, with the potential to bring a new treatment to patients who currently have no or inadequate options available. Synlogic is advancing the use of engineered strains of *E. coli* Nissle encoding phenylalanine ammonia lyase (PAL), an enzyme that breaks down Phe. By consuming dietary Phe in the GI tract and converting it to a harmless metabolite (TCA), treatment may be able to reduce plasma Phe levels. Synlogic's PKU development programme achieved proof-of-concept in 2021 based on an interim analysis from the Phase II Synpheny-1 clinical trial, with plans underway to initiate a Phase III trial in the first half of 2023.

### The broad potential applications of synthetic biotics

Based on their mechanisms of action and the clinically validated relationships between bacteria and the body's immune response, synthetic biotics

have the potential to treat a range of both metabolic and immunologic diseases. Some examples include HCU, EH and immunological diseases such as inflammatory bowel disease (IBD), Crohn's disease or ulcerative colitis.

Synlogic is currently advancing a clinical-stage programme in HCU, a rare metabolic disease caused by an inborn error of metabolism, specifically the loss of function of the enzyme cystathionine beta-synthase (CBS), which results in toxic buildup of the amino acid homocysteine and its metabolites in the blood and urine. People living with HCU are at risk of life-threatening strokes due to thromboembolism, lens dislocation, skeletal abnormalities and intellectual disability. Many patients are required to comply with a rigid methionine-restricted diet (methionine is a precursor to homocysteine) and available treatment options for HCU are limited due to efficacy and tolerability issues.

SYNB1353 is an orally administered, non-systemically absorbed drug candidate currently in Investigational New Drug (IND), enabling studies designed to reduce homocysteine levels by consuming methionine in the GI tract. Pre-clinical data has shown that SYNB1353 can lower blood homocysteine levels in non-human primates and mouse models.

Synlogic is also advancing the synthetic biotic SYNB8802 for the potential treatment of EH, a rare, chronic and progressive disease that is caused by a GI absorption disorder and can lead to recurrent kidney stones. With hyperoxaluria, oxalate crystals damage the kidneys and impair renal function, leading to severe pain, chronic kidney disease, end-stage renal disease and systemic oxalosis (the systemic accumulation of calcium oxalate). Some patients may ultimately need a kidney transplant or dialysis. There are currently no approved treatment options available.

SYNB8802 is an engineered strain of *E. coli* Nissle designed to convert excess oxalate to the harmless metabolite formate for excretion, thereby consuming oxalate in the GI tract and lowering urinary oxalate levels. In a Phase I study in healthy volunteers who were fed a high oxalate diet to increase urinary oxalate levels, SYNB8802 achieved proof-of-mechanism and demonstrated robust,

dose-dependent reductions in urinary and faecal oxalate with no serious or systemic adverse events.

Given the proof-of-concept and proof-of-mechanism achieved in Synlogic's PKU and EH programmes respectively, results thus far indicate that synthetic biotics could target other metabolites in the human GI tract that are dysregulated in immunological diseases. In one example, Synlogic is currently working to develop a synthetic biotic for the potential treatment of inflammatory bowel disease (IBD) as part of a research collaboration with Roche.

### Summary

In just a few years, Synlogic has advanced several promising synthetic biotics to clinical stage development and is anticipating multiple late-stage data readouts in the months ahead. Progress continues to validate the company's synthetic biotics platform with novel approaches in disease treatment that could bring new hope to patient communities living with serious diseases with unmet medical needs, including many that have only limited or sub-optimal treatment options. 📌



#### Dr Caroline Kurtz

Caroline joined Synlogic in October 2016 and is Chief Development Officer. She has 27 years of experience in the pharmaceutical industry and was previously vice president and GC-C

platform lead at Ironwood Pharmaceuticals. Prior to her role at Ironwood, she served as director of infectious diseases at GelTex/ Genzyme, where she led discovery of novel polymeric compounds as anti-infectives for intestinal and pulmonary infections. Caroline has been an author on more than 43 publications and has received several industry and academic honors for her work. She received her PhD in immunology from Harvard University in the laboratory of Dr John Weis and carried out post-doctoral training in viral immunology and central nervous system demyelinating diseases in the laboratory of Dr Robert Fujinami at the University of Utah. She received a BS in biochemistry from the University of New Hampshire.

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