

## Editorial

## Synthetic biology beyond borders.

Underlying every living cell, there is a beautifully concerted circuitry of signalling, metabolism and regulation that, together, orchestrate a variety of functions. Synthetic biology has targeted this circuitry at all levels, engineering it towards a wide array of applications – but also expanding our understanding fundamental aspects of the cell. Fuelled by advances in DNA sequencing, synthesis, editing, and computational modelling and circuit design, synthetic biology has broken tremendous ground in the last decade all across the world.

The same way engineers attempt to understand and create complex systems, biologists have strived to dissect and understand the complexities of biological systems. Bringing together both engineers and biologists with diverse backgrounds, the highly successful India-EMBO symposium ‘Engineering meets evolution: Designing biological systems’ was hosted at Chennai, India, in January 2020 – one of the last opportunities to gather in a physical meeting just before the COVID pandemic broke loose. Actually, the rapid and efficient rollout of vaccines designed to handle the COVID crisis is a very palpable example of how synthetic biology can have a positive impact on our daily lives (Brüssow, 2021). Born out of the exciting discussions during the symposium, ‘*Synthetic biology beyond borders*’, the current issue of *Microbial Biotechnology*, collates a number of interesting advances in the field from contributors all around the world. Composed by both review and research articles, this special issue covers topics ranging from efficient genome editing of cell factories to metabolic engineering for the production of complex, value-added molecules – with authors from 15 different countries in Europe, America and Asia. As synthetic biology continues to expand horizons, the importance of a special issue that covers a breadth of topics in the field cannot be overstated.

**Synthetic biology: moving biology from phenomenology towards engineering**

Ever since the first gene circuits were designed and constructed (Elowitz and Leibler, 2000; Gardner and Cantor, 2000), synthetic biology brought about an engineering approach to biology (Danchin, 2021) – whereby sophisticated behaviours can be designed into biological systems (de Lorenzo and Danchin, 2008; Stephanopoulos,

2012; Kahl and Endy, 2013; Church *et al.*, 2014; Flores Bueso and Tangney, 2017; Huang and Nikel, 2019; Wurtzel *et al.*, 2019). A wide range of switches, oscillators, logic gates, filters and even pulse generators have been incorporated into living cells under this conceptual framework. The defining aspect of synthetic biology is the rational design of biological circuitry using existing or novel genes, in a predictive fashion, with the ultimate goal of making biology more and more *engineerable* – where various standardized ‘parts’ and ‘modules’ can be reliably composed, physically and functionally, to yield ‘devices’ that display desired functionalities (Arkin, 2008; Silva-Rocha *et al.*, 2013; Decoene *et al.*, 2018). In this regard, synthetic biologists have to deal with an unavoidable aspect of living systems, that is, their capacity to evolve (Nørholm, 2019; Sambamoorthy and Sinha, 2019). As illustrated in a research article in this special issue, adaptive laboratory evolution is, on itself, a powerful tool for the design and optimization of cell factories. Evolution is an inherent property of every cell platform used for synthetic biology, and the plethora of *chassis* currently available supported the expansion of the range of practical applications (Danchin, 2012; Calero and Nikel, 2019; Abram and Udaondo, 2020). A research article in this special issue describes the genome and phenotypic characterization of yet another bacterial platform, *Pseudomonas umsongensis* GO16, a metabolically versatile *Pseudomonas* species. Synthetic microbial communities are likewise gaining increasing attention (Ibrahim and Raajaraam, 2021; Kumar *et al.*, 2021). These elements serve as the basis for the *Design–Build–Test–Learn* (DBTL) cycle that has become the cornerstone in the field, especially in the context of biofoundries (Holowko *et al.*, 2021) and some well-funded companies that are currently driving automation of synthetic biology. All these efforts are matched with the emerging role of artificial intelligence and deep learning – expected to make a major impact in the DBTL cycle, especially at the *Design* phase.

**The multiple fundamental and applied facets of synthetic biology**

When synthetic biology emerged as an independent scientific field, scientists devoted quite some effort in

developing tools for engineering living systems. This special issue includes articles that describe elegant examples of this sort, for example, synthetic promoters that respond to the availability of phosphorus, novel standard vectors for the efficient production of disulphide-containing proteins and CRISPR (clustered regularly interspaced short palindromic repeats) interference (CRISPRi) in *Pseudomonas*, a transposon toolbox supported by Cre/Lox recombination and counter-selection towards genome reduction, synthetic modulators of bacterial gene expression, and CRISPR/Cas9 and Cas12a-assisted protocols for genome engineering in filamentous fungi and photosynthetic bacteria. Computational synthetic biology has been an important cog in developing the field rapidly (Marchisio and Stelling, 2009) – making use of the rich toolbox indicated above. Iterative DBTL cycles, rooted in computational modelling, have enabled the construction of many a circuit, increasing the complexity and diversity of the gene circuitry architecture. Going forward, it will be vital to construct better genome-scale reconstructions and gene expression models, which could faithfully capture key interactions and cross-talk within the cell in order to more accurately reflect the performance of designer modules.

These endeavours require a deeper understanding of the general cell processes underlying microbial phenotypes. Such is the case of the 5'-regulatory regions present in DNA sequences (acting at the transcriptional level of regulation), ribonucleases (involved in post-transcriptional regulation) and catabolic repression (exerting effects at the metabolic, global level of regulation) – in addition to the redundancy in metabolic activities within central carbon metabolism. Four articles in our special issue discuss these aspects in the light of engineering regulatory traits in microbes.

Perhaps, the most industrially relevant application of synthetic biology lies in the development of microbes capable of synthesizing a variety of molecules, for example, drugs. A classic example is that of *Saccharomyces cerevisiae* engineered to produce artemisinic acid, the precursor of a key antimalarial drug (Ro *et al.*, 2006). The number and type of chemicals that can be produced with synthetic biology continue to expand (Prather, 2019), impacting not only research but also commercialization (Carbonell *et al.*, 2016; Martinelli and Nikel, 2019). In this issue, we have a number of research articles covering synthetic biology-guided metabolic engineering applications in a variety of microorganisms and target products, that is, *S. cerevisiae* (tyrosol, salidroside, vindoline and *cis,cis*-muconic acid), *Yarrowia lipolytica* (sesquiterpene, bisabolene and aromatic amino acids), *Pseudomonas putida* (5-ketofructose and vanillylamine), *Mycolicibacterium smegmatis* (steroids), *Streptomyces albus* (salinomycin), *Saccharopolyspora pogona*

(butenyl-spinosyn), *Shewanella oneidensis* (Pd/Ag and Pd/Au nanoparticles) and *Eubacterium limosum* (butyrate). These studies exploit a wide range of strategies to achieve improved titres and illustrate the utility of deploying toolboxes specifically tailored for different *chassis*. In addition, the state of the art in the metabolic engineering of amino acid and small peptide production is covered in two separate review articles. Programming of biosensors for accelerating cell factory optimization, sensing DNA damage and for general applications in environment, health and biomanufacturing is likewise discussed in three articles of this volume.

### Outlook: widespread adoption of synthetic biology beyond borders

While the benefits of synthetic biology outweigh the potential pitfalls, there are a number of key challenges that need to be surmounted in this decade, as synthetic biology goes more mainstream. The actual impact that scientific advances can have on the society involves social and political aspects in connection to adopting new technologies and how to use them in the context of responsible research and innovation (Gregorowius and Deplazes-Zemp, 2016). The overall perception of synthetic biology and its acceptance by consumers will be key to this end (Gallup and Ming, 2021). There are also regulatory challenges and safety concerns, which need to be dispelled by engaging scientists with the public and policymakers in a two-way dialogue and through proper education and diffusion of emerging technologies. We hope that such efforts will be shared by the global synthetic biology community beyond territorial and conceptual borders, especially when synthetic biology is scaled-up to offer global solutions (de Lorenzo and Marlière, 2016). Meanwhile, the examples collected in this special issue illustrate the current status of a burgeoning field that will continue to expand in the years to come and *Microbial Biotechnology* will continue to serve as a dynamic platform to communicate and discuss these developments.

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### Conflict of interest

None declared.

Karthik Raman<sup>1\*</sup> , Himanshu Sinha<sup>1\*\*</sup> ,  
Claudia E. Vickers<sup>2\*\*\*</sup>  and Pablo I. Nikel<sup>3\*\*\*\*</sup> 

<sup>1</sup>Department of Biotechnology, Centre for Integrative Biology and Systems Medicine (IBSE), Indian Institute of Technology Madras, Chennai, India; <sup>2</sup>CSIRO Future Science Platform in Synthetic Biology, Commonwealth Scientific and Industrial Research Organization (CSIRO), Dutton Park, Australia and <sup>3</sup>The Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Kongens Lyngby, Denmark

\*E-mail: kraman@iitm.ac.in

\*\*E-mail: sinha@iitm.ac.in

\*\*\*E-mail: claudia.vickers@csiro.au

\*\*\*\*E-mail: pabnik@biosustain.dtu.dk

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