SYNBIOCHEM Synthetic Biology Research Centre, Manchester — A UK foundry for fine and speciality chemicals production

Le Feuvre RA a,*, Carbonell P a, Currin A a, Dunstan M a, Fellows D a, Jervis AJ a, Rattray NJW a, Robinson CJ a, Swainston N a, Vinaixa M a, Williams A a, Yan C a, Barran P a, Breitling R a, Chen GG a, Faulon JL a, Goble C b, Goodacre R a, Kell DB a, Micklefield J a, Scrutton NS a, Shapira PC, Takano EA, Turner NJ a

a Manchester Centre for Synthetic Biology of Fine and Specialty Chemicals (SYNBIOCHEM), Manchester Institute of Biotechnology, Faculty of Life Sciences, University of Manchester, Oxford Road, Manchester, M13 9PT, United Kingdom
b SYNBIOCHEM, Data Lead, School of Computer Science, The University of Manchester, Manchester, M13 9PT, United Kingdom
c SYNBIOCHEM, Responsible Research and Innovation Lead, Alliance Manchester Business School, University of Manchester, Manchester, M13 9PT, United Kingdom

SYNBIOCHEM Synthetic Biology Research Centre, Manchester — A UK foundry for fine and speciality chemicals production

Le Feuvre RA a,*, Carbonell P a, Currin A a, Dunstan M a, Fellows D a, Jervis AJ a, Rattray NJW a, Robinson CJ a, Swainston N a, Vinaixa M a, Williams A a, Yan C a, Barran P a, Breitling R a, Chen GG a, Faulon JL a, Goble C b, Goodacre R a, Kell DB a, Micklefield J a, Scrutton NS a, Shapira PC, Takano EA, Turner NJ a

a Manchester Centre for Synthetic Biology of Fine and Specialty Chemicals (SYNBIOCHEM), Manchester Institute of Biotechnology, Faculty of Life Sciences, University of Manchester, Oxford Road, Manchester, M13 9PT, United Kingdom
b SYNBIOCHEM, Data Lead, School of Computer Science, The University of Manchester, Manchester, M13 9PT, United Kingdom
c SYNBIOCHEM, Responsible Research and Innovation Lead, Alliance Manchester Business School, University of Manchester, Manchester, M13 9PT, United Kingdom

ARTICLE INFO

Article history:
Received 21 June 2016
Received in revised form 8 July 2016
Accepted 11 July 2016

ABSTRACT

The UK Synthetic Biology Research Centre, SYNBIOCHEM, hosted by the Manchester Institute of Biotechnology at the University of Manchester is delivering innovative technology platforms to facilitate the predictable engineering of microbial bio-factories for fine and speciality chemicals production. We provide an overview of our foundry activities that are being applied to grand challenge projects to deliver innovation in bio-based chemicals production for industrial biotechnology.

© 2016 The Authors. Production and hosting by Elsevier B.V. on behalf of KeAi Communications Co. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
learning and informed re-design. This unique capability of SYN-BIOCHEM offers a comprehensive suite of platform technologies for focused grand challenge projects.

The Centre’s Design platform is developing a suite of in silico tools to support the design of synthetic metabolic pathways [25]. These tools cover enzyme selection, design of reusable genetic parts (including ribosome binding site and codon optimisation), intelligent sampling of combinatorial design space through a Design of Experiments approach, and support of pathway assembly methods. This work builds on our strong foundation in the development of genome mining tools, such as antiSMASH [26], and automated pipelines for cheminformatics and biosynthetic pathway prediction, such as RetroPath and related tools [27–30]. The recent development of SensiPath, for the enhanced design of metabolic pathways with intermediates that are detectable by biosensors and therefore amenable to high-throughput screening, further expands this growing arsenal of design tools [31]. Underpinning much of these design approaches is expertise in systems biology and metabolic modelling [32–34], allowing in silico simulations to drive each iteration of the Design-Build-Test-Learn cycle. Throughout this work, the Centre is committed to the support of and further development of community standards including SBOL [35] and SBOL Visual [36].

Based on the resulting design blueprints, the Build platform is responsible for the manufacturing of catalytic, regulatory and structural parts; assembly of these parts into pathways, circuits and microbial scaffolds; and engineering of the chassis for efficient biosynthesis of target compounds with high product yields. We have automated our DNA assembly methods including the efficient construction of directed evolution libraries, that allow the rapid optimization of the enzyme building blocks of our synthetic pathways (SpeedyGenes [20]), and are currently developing more efficient selective library generation methods. Growth of our collection of structural, regulatory and bio-catalytic parts is benefiting from automated high-throughput (HTP) preparation and quality control. We are harnessing our new robotics platforms for automated combinatorial pathway assembly, chassis growth and target production (media dispensing, colony picking, and automated HTP growth, through to automated metabolite and compound extraction), to rapidly assemble and screen diverse part libraries and biochemical pathways, and to phenotypically characterize our engineered microbial systems. We are developing a regulatory and biosensor toolkit to facilitate real-time control, sensing and quantification of specific products, intermediates or cofactors from engineered pathways in living cells, enabling high-throughput screening and selection. Novel riboswitches are being constructed from target-specific aptamers (identified through our automated SELEX platform), building on our experience in developing orthogonal riboswitches as gene regulatory tools [37]. Pathway-specific biosensors are also being constructed using sensitive transcription factor/operator pairs identified through the mining of next-generation transcriptome sequencing data. Picodroplet (SphereFluidics) and fermentation platforms provide the Centre with capabilities for microbial production and analysis from single cell level through to multi-litre scale. Optimisation at the laboratory scale will inform further collaborative development and evaluation at larger pilot scales.

Our Test platform, built on a wealth of expertise in targeted analytics and untargeted metabolomics [38,39], is utilising an integrated suite of high-throughput screening methods for the quantification of optimised target compound production, assayng target metabolites and enzymes, as well as tracking pathway intermediates and undesired side products or potential metabolic bottlenecks. Our comprehensive state-of-the-art targeted and untargeted mass spectrometry platforms are being applied for
metabolomic profiling of molecular species of interest, and a dedicated ion mobility platform provides additional discriminative power for compound identification. We are signal processing and deconvolving our data using a range of open source tools, including our evolving mzMatch toolbox [40], to provide rapid information to inform the iterative re-design, debugging and optimisation of our production strains. Our dedicated integrated infrastructure provides seamless forward and backward data traceability across analysed experimental data and models. We are using an iterative active learning strategy for experimental planning to ensure the generation of high-quality training sets that are used at each iteration to refine and update the models. The Centre is applying machine-learning workflows trained on the analysed data to learn sequence- and structure-activity relationships [22,28] that feed back into the Design platform. Similarly, a growing catalogue of predictive tools that learn from resulting data is applied to the redesign of additional SynBio components, from chemical diversity discovery to strain and process optimization [25,41].

Our integrated Design—Build—Test platforms are being applied to a selection of “grand challenge” projects that target key chemical scaffolds including: Alkaloids, which constitute a vast range of plant-derived compounds with potent bioactivities and are a source of numerous drugs and drug precursors [42]; Flavonoids, traditionally of plant origin with many desirable properties and used as antibacterial, antitoxin, antiviral and antifungal agents [43]; and Terpenoids, a valuable class of molecules with a range of uses from flavours and fragrances to antimalarial drugs and biofuel precursors [44]. SynBio-derived chemical ingredients have already reached the food and cosmetics market, with companies now focusing on the production of purified fine chemicals (e.g. nootkatone fragrance) which once extracted are considered as “natural” and do not require labelling of the resulting product as containing genetically modified organisms. It is, however, early days and the development of consumer responses to more widespread use of SynBio ingredients remains to be seen [45]. Pre-market regulation of SynBio products and specifically the production processes that use synthetically modified organisms (SMO’s) will need to be carefully considered to ensure continued public acceptance. The recent development of three official Opinion documents by the Scientific Committees of the European Commission on potential risks of SynBio and the associated research needs [46–48] is an important contribution to this process.

Moreover, complementing our core science programmes, SYN-BIOCHEM’s Responsible Research and Innovation (RRI) platform is developing major programmes on the societal aspects faced by SynBio, including real-time assessment and anticipation of research and innovation trajectories, deliberation and reflection on emerging ethical, regulatory and policy issues, and collaborative research development. SYN-BIOCHEM’s RRI platform seeks to initiate early multiway dialogue, provide expertise, guidance and training in the responsible governance of SynBio innovation, and foster public engagement and training for the research community, in order to anticipate, prepare for and if necessary mitigate the impacts of SynBio technology in the wider society, economy and environment [49]. Examples of the Centre’s activity in this domain include contributions to European Commission reports on SynBio
safety issues [50], involvement in establishing a good practice framework – The www.Responsibility-Navigator.eu [51], and the publication of a comprehensive sociological analysis of SynBio as an emerging scientific field [52].

In summary, SYNBIOCHEM is addressing major SynBio challenges, employing its foundry concept and integrated technology platforms to facilitate the predictable engineering of microbial biofactories for chemical production. Through its open collaborative ethos the Centre is proactively engaging with academic and industrial partners, not only in the UK but also on the international stage. Development of industrial collaborations, foresight and awareness is a major focus of the Centre. We are accelerating the commercial translation of our activities through continued dialogue with industrial partners that is enabled through joint co-funded research projects, that target and apply our technologies to commercially relevant chemicals at an early stage (e.g. a collaboration with DSM on a single step fermentative production route for pravastatin [13]), industry funded PhD studentships and close partnerships with instrument developers. Building on our existing productive UK and EU collaborations, the Centre is working closely with the other UK SBRCs with joint projects, workshops and events, whilst international partnerships are providing broader access to technological and expertise. Examples are UK research council co-funded partnership awards with China and Japan, collaboration awards with Brazil and Malaysia, and a steady influx of international PhD students who join the MIB and the University of Manchester. Further information about the Centre and routes to collaboration can be obtained from the SYNBIOCHEM website: http://synbiochem.co.uk.

Acknowledgement

This is a contribution from the Manchester Centre for Synthetic Biology of Fine and Speciality Chemicals (SYNBIOCHEM) and acknowledges the Biotechnology and Biological Sciences Research Council (BBSRC) and Engineering and Physical Sciences Research Council (EPSRC) for financial support (Grant No. BB/M017702/1).

References

[1] A synthetic biology roadmap for the UK, http://www.rcuk.ac.uk/publications/reports/syntheticbiologyroadmap [accessed 21.06.16].
[2] Biodesign for the bioeconomy UK synthetic biology strategic plan. 2016. https://connect. innovatenuk.org/documents/2826135/31405930/Biodesign-for-the-Bioeconomy-2016-DIGITAL-updated-21_03_2016. pdf [accessed 21.06.16].
[3] Kid A, Wyke T. editors. Manchester: Making the Modern City, Liverpool University Press; 2016.
[4] Weizmann C, Rosenfeld B. The activation of the butanol-acetone fermentation of carbohydrates by Clostridium acetobutylicum. Biochem J 1937;31(4): 619–39.
[5] Furter WF. A century of chemical engineering, Plenum Press (NY & London); 1980. ISBN 0-306-40895-3. https://en.wikipedia.org/wiki/History_of_chemical_engineering [accessed 21.06.16].
[6] Industrialisation of Biology. A roadmap to accelerate the advanced manufacturing of chemicals, National Academies Press (US); 2015; Jun. http://dx.doi.org/10.17226/19001.
[7] Padden CJ, Keasling JD. Semi-synthetic artemisinins: a model for the use of synthetic biology in pharmaceutical development. Nat Rev Microbiol 2014;12: 355–67.
[8] Galanie S, Thodey K, Trenchard IJ, Interanne MF, Smolke CD. Complete biosynthesis of opioids in yeast. Science 2015;349(6252):1095–100.
[9] Smanski MJ, Zhou H, Claesen J, Shen B, Fischbach MA, Voigt CA. Synthetic biological platforms for renewable production of chemicals. Proc Natl Acad Sci USA 2015;112:2847–52.
[10] Marwick HS, Cheaigh AH, Tait S, Mansell DJ, Jervis A, Lygidakis A, et al. Enzymatic menthol production: one-pot approach using engineered Escherichia coli. ACS Synth Biol 2015;4:1112–23.
[11] Menon N, Pásztor A, Menon BRK, Kalilo P, Fisher K, Akhtar MK, et al. A microbial platform for renewable propane synthesis based on a fermentative butanol pathway. Biotechnol Biofuels 2015;8:61.
[12] Wu MC, Lowe PT, Robinson CJ, Vincent HA, Dixon N, Leigh J, et al. Rational re-engineering of a transcriptional silencing PreQ1 riboswitch. J Am Chem Soc 2015;137:9015–21.
[13] Payne KA, White MD, Fisher K, Khara B, Bailey SS, Parker D, et al. New cofactor supports α,β-unaturated acid decarboxylation via 1,3-dipolar cycloaddition. Nature 2015;522:497–501.
[14] White MD, Payne KA, Fisher K, Marshall SA, Parker D, Ratnay NJ, et al. UbX is a flavin prenyltransferase required for bacterial ubiquinone biosynthesis. Nature 2015;522:502–6.
[15] Muttig FG, Knauss T, Scrutton NS, Breuer M, Turner NJ. Conversion of alcohols to enantiopure amines through dual–enzyme hydrogen-hydrogenocarboxylating cascade. Science 2015;349:1525–9.
[16] Currin A, Swanston N, Day PJ, Kell DB. SpeedyGenes: an improved gene synthesis method for the efficient production of error-corrected, synthetic genes. Nucleic Acids Res 2014;42:W395–400.
[17] Currin A, Swanston N, Day PJ, Kell DB. Synthetic biology for the directed evolution of protein biocatalysts: navigating sequence space intelligently. Chem Soc Rev 2015;44:1172–239.
[18] Kell DB, Swanston N, Pir P, Oliver SG. Membrane transporter engineering in industrial biotechnology and whole cell biocatalysis. Trends Biotechnol 2015;33(4):237–46.
[19] Carbonell P, Currin A, Dunstan M, Fellows D, Jervis A, Ratnay NJ, et al. SYN- BIOCHEM-a SynBio foundry for the biosynthesis and sustainable production of fine- and speciality chemicals. Biochem Soc Trans 2016;44(3):675–7.
[20] Carbonell P, Currin A, Jervis AJ, Ratnay NJ, Swanston N, Yan C, et al. Bioinformatics for the synthetic biology of natural products: integrating across the Design-Build-Test cycle. Nat Prod Rep 2016;33(8):925–32.
[21] Wu MC, Bliu K, Duddridge S, Krug D, Kim HJ, Kell DB, et al. AntimS3: a comprehensive resource for the genome mining of biosynthetic gene clusters. Nucleic Acids Res 2015;43(W1):W237–43.
[22] Carbonell P, Parutto F, Baudier C, Junot C, Faulon JL. Retropath: automated pipeline for embedded metabolic circuits. ACS Synth Biol 2014;3:437–75.
[23] Mellor J, Grigoras I, Carbonell P, Faulon JL. Semisupervised gaussian process for automated enzyme search. ACS Synth Biol 2016;5(6):518–28.
[24] Swanston N, Hastings J, Dekker A, Muthukrishnan V, May J, Steinbeck C, et al. UBiCHEB: an API for accessing the CHEBI database. J Cheminform 2016;8:11.
[25] Hastings J, Owen G, Dekker A, Ennis M, Kale N, Muthukrishnan V, et al. CheBI in 2016: improved services and an expanding collection of metabolites. Nucleic Acids Res 2016;44(D1):D214–9.
[26] Deininger B, Libis V, Carbonell P, Faulon JL. SensiPath: computer-aided design of sensing-enabling metabolic pathways. Nucleic Acids Res 2016;44(41):W226–31.
[27] Swanston N, Mendes P, Kell DB. An analysis of a ‘community-drivers’ recon- struction of the human metabolic network. Metabolism 2013;9(4):757–64.
[28] Swanston N, Smallbone K, Hefi Z, Dobson PD, Brewer J, Hanscho M, et al. Recon 2.2: from reconstruction to model of human metabolism. Metabolites 2016;6:129.
[29] Stanford NJ, Millard P, Swanston N. RoboKoD: microbial strain design for (over)production of target compounds. Front Cell Dev Biol 2015;3:17.
[30] Rohner N, Beal J, Clancy K, Bartley B, Misirli G, Grünberg R, et al. Sharing structure and function in biological design with SBOL 2.0. ACS Synth Biol 2016;5(6):498–506.
[31] Quinn JY, Cox RS, Adler A, Beal J, Bhatare S, Cai Y, et al. SBOL: a graphical format, java library, R library, and tool-chain for mass spec- trometry. Analyst 2016;141(8):351–60.
[32] Swainston N, Mendes P, Kell DB. An analysis of a ‘community-drivers’ recon- struction of the human metabolic network. Metabolism 2013;9(4):757–64.
[33] Swanston N, Smallbone K, Hefi Z, Dobson PD, Brewer J, Hanscho M, et al. Recon 2.2: from reconstruction to model of human metabolism. Metabolites 2016;6:129.
[34] Stanford NJ, Millard P, Swanston N. RoboKoD: microbial strain design for (over)production of target compounds. Front Cell Dev Biol 2015;3:17.
[35] Rohner N, Beal J, Clancy K, Bartley B, Misirli G, Grünberg R, et al. Sharing structure and function in biological design with SBOL 2.0. ACS Synth Biol 2016;5(6):498–506.
[36] Quinn JY, Cox RS, Adler A, Beal J, Bhatare S, Cai Y, et al. SBOL: a graphical format, java library, R library, and tool-chain for mass spec- trometry. Analyst 2016;141(8):351–60.
[37] Schelter RA, Jankevics A, Jansen RC, Swertz MA, Brettling R. PeakML/mzMatch: a file format, java library, R library, and tool-chain for mass spec- trometry data analysis. Anal Chem 2011;83(7):2786–93.
[38] Pasotti L, Zucca S. Advances and computational tools towards predictive design in biological engineering. Comput Math Methods Med. Hindawi Pub- lishing Corp; 2014; p. 369681.
[39] Cusinelli TP, Cusinelli B, Lamb AJ. Alkaloids: an overview of their antibacterial, antibiotic-enhancing and antimicrobial activities. Int J Antimicrob Agents
Friedman M. Overview of antibacterial, antitoxin, antiviral, and antifungal activities of tea flavonoids and teas. Mol Nutr Food Res 2007;51:116–34.

Bicas JL, Dionisio AP, Pastore GM. Bio-oxidation of terpenes: an approach for the flavor industry. Chem Rev 2009;109(9):4518–31.

Hayden EC. Synthetic-Biology firms shift focus. Nature 2014;505:598.

European Commission. Opinion on synthetic biology I definition Luxembourg. 2014. http://dx.doi.org/10.2772/76553.

European Commission. Opinion on Synthetic Biology II Risk assessment methodologies and safety aspects. 2015. http://dx.doi.org/10.2772/63529.

European Commission. Final Opinion on Synthetic Biology III: risks to the environment and biodiversity related to synthetic biology and research priorities in the field of synthetic biology. 2015. http://dx.doi.org/10.2875/590512.

Li Y, Shapira P. Synthetic biology: reshaping the future? PLOS Synbio Blog 2016. 26 Jan, http://blogs.plos.org/synbio/2016/01/26/synthetic-biology-reshaping-the-future-manchester-policy-workshop-considers-implications-of-synbio-by-yanchao-li-and-philip-shapira/ [accessed 21.06.16].

Breitling R, Takano E, Gardner TS. Judging synthetic biology risks. Science 2015;347(6218):107.

Kulmann S, Edler J, Ordóñez-Matamoros G, Randles S, Walhout B, Gough C, et al. The Res-AGorA EU FP7 funded project. http://responsibility-navigator.eu/; 2016 [accessed 21.06.16].

Balmer AS, Bulpin K, Molynes-Hodgson S. Synthetic Biology: a sociology of changing practices. Basingstoke. MacMillan: Palgrave; 2016.