Synthetic spatial patterning in bacteria: advances based on novel diffusible signals

Martina Oliver Huidobro,† Jure Tica,‡ Georg K. A. Wachter and Mark Isalan*§
Department of Life Sciences, Imperial College London, London, SW7 2AZ, UK.

Summary

Engineering multicellular patterning may help in the understanding of some fundamental laws of pattern formation and thus may contribute to the field of developmental biology. Furthermore, advanced spatial control over gene expression may revolutionize fields such as medicine, through organoid or tissue engineering. To date, foundational advances in spatial synthetic biology have often been made in prokaryotes, using artificial gene circuits. In this review, engineered patterns are classified into four levels of increasing complexity, ranging from spatial systems with no diffusible signals to systems with complex multi-diffusor interactions. This classification highlights how the field was held back by a lack of diffusible components. Consequently, we provide a summary of both previously characterized and some new potential candidate small-molecule signals that can regulate gene expression in Escherichia coli. These diffusive signals will help synthetic biologists to successfully engineer increasingly intricate, robust and tuneable spatial structures.

Introduction

Biological patterning can be defined as the organized arrangement of an organism’s features (Davies, 2017), where an initially uniform field of cells gains complexity and heterogeneity in the spatial domain (Murray, 2013; Davies and Glykofrydis, 2020). This structure is crucial for function in multicellular organisms (Blest, 1957; Stevens et al., 2006; Strauss et al., 2020).

Understanding the mechanisms behind patterning is difficult due to the tangled nature of biology. The study of developmental biology largely consists in observing embryos or tissues and in perturbing the systems to validate different hypotheses (Murray et al., 2011; Rappoport et al., 2014). This approach leads to insights into the complexity of a particular biological system. In contrast, in synthetic biology a system is built from first principles, making it simpler, more controllable and insulated from the natural genetic context (Nielsen et al., 2016; Meyer et al., 2019). Building the desired patterns with a synthetic system is one step towards showing that these basic principles can potentially occur in biology (Davies, 2017; Luo et al., 2019; Santos-Moreno and Schaerli, 2019). However, it is important to acknowledge that successful engineering does not necessarily imply the occurrence of specific mechanisms in natural systems. Synthetic patterning systems nonetheless provide powerful tools for bioengineering and offer a proof that these mechanisms could potentially occur in development.

In addition to expanding our knowledge of developmental biology, building a simple and programmable system is necessary for the synthesis of patterned tissues, organoids or biofilms for downstream biotechnology applications (Scholes and Isalan, 2017; Davies and Glykofrydis, 2020). To work towards this goal, bacteria provide a relatively simple chassis, where spatial systems can be built in a controlled manner from first principles, using modelling to guide engineering (Elowitz and Leibler, 2000; Gardner et al., 2000; Salis et al., 2009).

This review outlines the progress in synthetic patterning using Escherichia coli and focuses on how the field was historically held back by a lack of diffusible components. It then highlights recently characterized components that can be used to build more complex, multi-diffusor systems. While interesting patterning systems were also engineered in other cellular systems (Cachat et al., 2017; Sekine et al., 2018; Tordoff et al., 2021), this review focuses on E. coli.
**Engineered circuits for spatial patterning**

To consider the problem of synthetic patterning systems systematically, here we suggest grouping them into four levels according to their design characteristics (Fig. 1). Level 0 circuits do not contain any synthetic signals that diffuse through normal Fickian diffusion; instead, spatial structure emerges by other processes, such as cellular growth. Level 1 systems rely on one or more diffusing components whose production is not dynamically regulated by the circuit. Level 2 systems incorporate a single diffusible component, which is dynamically regulated by the circuit components. Level 3 systems use multiple dynamically regulated diffusible components.

Spatial systems where the diffusing components are not dynamically regulated by the circuit (Level 1) were the first to be engineered in *E. coli* (Basu et al., 2005). Stripe patterning systems are the most prominent example among Level 1 circuits. Initially, diffusor gradients were interpreted by intracellular incoherent feedforward circuits, to form rings of gene expression at intermediate distances from their source (Basu et al., 2005; Schaerli et al., 2014; Kong et al., 2017). Bistable mutually inhibitory circuits were also used to interpret morphogen gradients, forming systems that can robustly generate sharp boundaries between two or more spatial regions of gene expression (Barbier et al., 2020; Grant et al., 2020).

Hierarchical patterning is a scalable Level 1 system, implemented in a recently engineered circuit in *E. coli* (Boehm et al., 2018). Two diffusor sources at the edges of a spatial domain are interpreted by an AND gate circuit to lead to three distinct spatial partitions. In theory, additional diffusing species and AND gates could be introduced to generate increasingly complex structures: for example (2^n - 1) spatial domains could be generated.

![Fig. 1. Four levels of regulatory complexity in engineered spatial patterning systems.](image-url)

© 2021 The Authors. *Microbial Biotechnology* published by Society for Applied Microbiology and John Wiley & Sons Ltd., *Microbial Biotechnology, 15*, 1685–1694
from $n$ orthogonal diffusing signals in a one-dimensional space. While this type of patterning can explain some developmental patterning programs, such as that of the vertebrate neural tube (Briscoe and Small, 2015), it fails to capture self-organizing periodic structures, such as digit patterning in the chick limb bud (Sheth et al., 2012; Raspopovic et al., 2014).

Spatial patterns are often shaped by the complex interaction of the circuit components with cellular growth and other biological and physical properties of the system. This interplay is highlighted in some recently engineered Level 0 systems, where a synchronized circuit of the repressilator (Elowitz and Leibler, 2000) was used to produce periodic concentric ring patterns in growing colonies of cells, in the absence of diffusing signals (Potvin-Trottier et al., 2016; Riglar et al., 2019). The mechanism that provides spatial structure consists of a combination of growth at the edge of the bacterial colony, and of the arrest of circuit activity at the colony interior.

Reaction–diffusion systems, where the diffusor is dynamically regulated by the circuit components (Level 2), have also been engineered successfully (Danino et al., 2010; Payne et al., 2013). Unlike Level 1, Level 2 systems generally do not rely on pre-patterns or positional information. For this group, engineering was mainly focused on circuits with a self-activating and a laterally inhibiting component. A prominent example is the oscillator with a diffusible positive feedback, observed to generate spatially synchronized oscillations and propagating waves (Danino et al., 2010). A further example with a diffusible inhibiting component, but lacking a diffusible positive feedback, was shown to produce a ring pattern in growing colonies of cells, which is not reliant on diffusor gradients (Payne et al., 2013; Cao et al., 2016). All the systems mentioned above rely on a single diffusive component; the engineering of these systems becomes increasingly challenging for more diffusing species.

While numerous successes were achieved with Level 1 and 2 spatial circuits, successfully engineering Level 3 systems, consisting of multiple dynamically regulated diffusors, is still in its infancy. Turing patterns are the most prominent example of Level 3 systems; they are formed by reaction–diffusion circuits of at least two diffusors, where generally the first is self-activating, whereas the second performs a lateral inhibition (Turing, 1952; Gierer and Meinhardt, 1972; Scholes et al., 2019). Classical, deterministic Turing patterns self-organize into periodic spot, stripe or labyrinthine spatial structures (Horváth et al., 2009; Asakura et al., 2011; Murray, 2013). Originally, they were formulated mathematically with little regard to biological context (Turing, 1952). Computationally, many biological candidate networks were found to produce Turing patterns (Marcon et al., 2016; Zheng et al., 2016; Scholes et al., 2019). However, engineering them remains difficult mainly because of their high sensitivity to changes in system parameters (Maini et al., 2012; Scholes et al., 2019). The issue of fine-tuning is exacerbated by the lack of appropriately tuneable components to achieve the narrow parameter space in which classical Turing patterns occur.

Greater success was seen with stochastic Turing patterns because their fine-tuning requirements are more relaxed (Butler and Goldenfeld, 2011). Stochastic Turing patterns were recently engineered in E. coli with a circuit implemented according to the self-activation and lateral inhibition topology, with two diffusible quorum-sensing signals (Karig et al., 2018). While easier to engineer, stochastic Turing patterns display more irregularity in their periodic spatial structure (Butler and Goldenfeld, 2011; Karig et al., 2018). Solitary structures are another possible mechanism for periodic patterning due to their close resemblance to some natural patterns (Sekine et al., 2018). They can also be formed by activator–inhibitor reaction–diffusion systems; however, their fine-tuning requirements are more relaxed compared to Turing patterns, and might therefore be easier to build (Koga and Kuramoto, 1980; Purwins et al., 2010). Even though they are still an unsolved engineering problem, solitary patterns were recently observed in a refactored Nodal–Lefty system in HEK cells (Sekine et al., 2018).

While elusive in synthetic biology, regular-repeat Turing patterns were more readily observed in chemical reaction systems, where they were first detected in the early 1990s in the chlorite–iodide–malonic acid (CIMA) reaction (Castets et al., 1990; Lengyel et al., 1993). Turing patterns were then also discovered in the thiourea–iodate–sulfite (TuIS) reaction with a rational design approach (Horváth et al., 2009). Unlike biological systems, chemical reactions are reliably described by the simpler laws of mass action, and system parameters can often be identified (Turányi, 1994; Kügler et al., 2009; Pušnik et al., 2019; Yeoh et al., 2019; Tica et al., 2020). Furthermore, the tuning of these systems by changing initial reactant concentration or temperature is easily achieved and has predictable effects on the dynamics of the system (Horváth et al., 2009; Carballido-Landeira et al., 2010; Asakura et al., 2011). Lastly, the systems are easily isolated from external interacting components; this is difficult to achieve with biological systems where cross-talk between synthetic parts and with the cellular chassis is inevitable (Ceroni et al., 2015; Nielsen et al., 2016; Butzin and Mather, 2018; Müller et al., 2019; Du et al., 2020). These and other related factors made chemical reaction systems suited to support such a fine-tuned phenomenon as Turing patterns. However, recent advances with the
parametrization of synthetic genetic circuits may open new possibilities also in the field of synthetic biology (Espah Borujeni et al., 2020).

Multi-diffusor systems were historically held back by the lack of a diverse palette of well-characterized diffusible components. Studies tried to circumvent this shortage, for example, by considering the E. coli cell chassis to be one of the diffusors (Duran-Nebreda et al., 2021). However, cell growth within a bacterial colony differs significantly from classical diffusion because it is not directionally unbiased. The movement of cells in space is limited within a colony and mainly happens outwards, in the direction of growth. Due to developments in directed evolution, genome mining and metabolic engineering, more well-characterized diffusible components have recently become available (Meyer et al., 2019; Du et al., 2020). We expect these advances to be pivotal in the further development of spatial patterning systems, particularly of multi-diffusor circuits.

The same diffusible synthetic components were also used in the engineering of spatially distributed computing systems, where neighbouring bacterial colonies containing simple logic gate circuits communicate by secreting diffusible signals (Tamsir et al., 2011; Du et al., 2020). Spatially distributed systems enable complex biological computation ranging from basic logic operations (Du et al., 2020) to more complex neural-like computing (Li et al., 2021). These systems do not fall in any of the circuit categories introduced in this study due to their spatially distributed nature. Even though outside the scope of this article, these types of systems would also directly benefit from the development of novel well-characterized signalling modules.

### Novel diffusible components

Historically, the biggest hindrance to the development of spatial systems with two or more dynamically regulated diffusors is the lack of well-characterized, robust and tuneable diffusing components for E. coli (Scholes and Isalan, 2017). The basic criteria that synthetic signalling modules need to satisfy are: (i) diffusion and bidirectional passage across cellular boundaries; (ii) ability to regulate gene expression; (iii) simple synthesis pathways in E. coli, to avoid metabolic burden and issues with refactoring overly complex systems; (iv) orthogonality to other synthetic components and endogenous E. coli chemistry; (v) it is also desirable that the signals are well-characterized and optimized for model-based rational engineering.

Among potential diffusible components, quorum-sensing homoserine lactones (HSLs) are most widely used in E. coli synthetic biology (Basu et al., 2005; Danino et al., 2010; Karig et al., 2018). HSLs are well-studied and were recently reviewed in the context of synthetic biology and pattern engineering (Papenfort and Bassler, 2016; Boo et al., 2021). While being versatile and easy to implement, they also possess limitations, which mainly stem from their similar chemistry. First, even though orthogonal HSLs exist, cross-talk between them is common (Boedicker and Nealson, 2016; Silva et al., 2017; Tekel et al., 2019; Du et al., 2020). In addition, engineering differential diffusion with pairs of HSLs can be challenging; this is of particular interest for Turing pattern engineering and could also be of interest with other spatiotemporal systems where space scale separation is needed (Lengyel and Epstein, 1992; Szalai and De Kepper, 2008; Horváth et al., 2009). While quorum sensing is a highly effective solution to implement cell–cell communication in prokaryotes, this article aims to move beyond it and focus on novel non-quorum-sensing signals.

Recently, 12 different small molecule inducible genetic systems were optimized for use in E. coli synthetic biology (Meyer et al., 2019). These were incorporated in the ‘Marionette’ strain, which provides the capability of regulating 12 genes simultaneously and independently. However, to use these inducible systems in a Level 2 or 3 spatial circuit, the small molecules must be produced endogenously from freely available precursors. Among the Marionette components, at least six could potentially be easily produced by E. coli with enzymes ported from other microorganisms: excluding quorum-sensing systems, these are DAPG, salicylate, protocatechuate, naringenin, vanillate, acrylate).

These avenues were further explored in a recent study where six novel, orthogonal, small-molecule inducers were developed for use in E. coli synthetic biology (Du et al., 2020). Both their inducible genetic components and synthesis mechanisms were developed and optimized for synthetic cell–cell communication. A screen of the literature shows that many more diffusible signals could be ported to E. coli, as candidates for well-behaving signalling modules. Table 1 provides a list of the recently discovered signals and of the potential candidates. Although this review focuses on E. coli, some studies indicate that these diffusors may be ported to other prokaryotes as well as some eukaryotes for a wider range of applications. For instance, three of the molecules in Table 1 have successfully been engineered in E. coli, S. cerevisiae and mammalian cells (HEK-293T) (Du et al., 2020).

For the successful implementation of these signals, it is important to optimize both the synthetic and the sensing components, where the efficiency in the endogenous synthetic system should meet the sensitivity of the sensing component. For example, it could easily happen that the endogenous synthetic mechanisms do not produce
| Component          | Pubchem ID | Synthesis mechanism | Degradation  | Receptor | Max fold induction | Molecular weight (Da) | Diffusion rate (mm² h⁻¹) | References                                                                                      |
|--------------------|------------|---------------------|--------------|----------|--------------------|-----------------------|------------------------|-----------------------------------------------------------------------------------------------|
| **Novel diffusible signals optimized for synthetic biology** |            |                     |              |          |                    |                       |                        |                                                                                |
| DAPG               | 16547      | phlACBD             | phlG         | phlF     | 1380               | 210.18                | 2.66                   | Bottiglieri and Keel (2006), Meyer et al. (2019), Du et al. (2020)                        |
| Salicylate         | 338        | pchBA/irp9/ybts     | nahG         | nahR     | 47                 | 138.12                | 3.22                   | Meyer et al. (2019), Du et al. (2020)                                                      |
| p-Coumaroyl-HSL   | 71311837   | rpaI, 4cl, tal      | aiiA         | rpaR     | 170                | 247.25                | 2.47                   | Liao et al. (2018), Du et al. (2020)                                                       |
| Isovaleryl-HSL     | 71627311   | bjal, bkdhFGH, ipdA1| aiiA         | bjaR     | 350                | 185.22                | 2.82                   | Liao et al. (2018), Du et al. (2020)                                                       |
| MMF                | N/A        | mmlLHP              | N/A          | mmfL     | 26                 | 198.22                | 2.73                   | Du et al. (2020)                                                                            |
| Naringenin         | 932        | chs, chi, 4cl, tal  | fns          | fdeR     | 16                 | 272.25                | 2.37                   | Lee et al. (2015), Du et al. (2020)                                                        |
| C₆-HSL            | 10130163   | rhlI                | aiiA         | rhlR     | 124                | 171.2                 | 2.92                   | Du et al. (2020)                                                                            |
| 3OC₆-HSL          | 71914579   | cepI, 4cl, tal      | aiiA         | cepR     | 150                | 227.3                 | 2.57                   | Du et al. (2020)                                                                            |
| Cumate             | 10820      | N/A                 | N/A          | cymR     | 860                | 164.2                 | 2.97                   | Meyer et al. (2019)                                                                        |
| Vanillate          | 8468       | tal, c3h, cont, fcs, ech | fns          | fdeR     | 16                 | 272.25                | 2.37                   | Ni et al. (2015), Wu et al. (2018), Meyer et al. (2019)                                      |
| IPTG               | 656894     | N/A                 | N/A          | lacI     | 688                | 238.3                 | 2.51                   | Meyer et al. (2019)                                                                        |
| ATC                | 54675785   | N/A                 | N/A          | tetR     | 490                | 426.4                 | 1.95                   | Meyer et al. (2019)                                                                        |
| L-arabinose        | 439195     | N/A                 | N/A          | araC/araE| 500                | 150.13                | 3.10                   | Meyer et al. (2019)                                                                        |
| Protocatechuate    | 6209       | N/A                 | N/A          | betI     | 306                | 139.62                | 3.20                   | Meyer et al. (2019)                                                                        |
| 3OH-C₄-HSL        | 11681427   | cinI                | aiiA         | cinR     | 500                | 327.46                | 2.19                   | Meyer et al. (2019)                                                                        |
| Acrylate           | 6581       | aspA, panD, act, acl2, yciA | N/A          | acuR     | 84                 | 72.06                 | 4.39                   | Meyer et al. (2019), Ko et al. (2020)                                                      |
| Erythromycin       | 12560      | N/A                 | N/A          | mphR, ery| 37                 | 733.9                 | 1.56                   | Zhang et al. (2010), Meyer et al. (2019)                                                   |
| **Potential diffusible signals for synthetic biology** |            |                     |              |          |                    |                       |                        |                                                                                |
| Kynurenine         | 846        | kynU                | kynR         | kynU     | 208.21             | 246.4                 | 2.67                   | Kurnasov et al. (2003), Hanko et al. (2020)                                                |
| Itaconate          | 811        | cadA                | ripABC       | itcR     | 215                | 130.1                 | 3.31                   | Okamoto et al. (2014), Hanko et al. (2018, 2020), Barbier et al. (2020)                    |
| Acetoin            | 179        | budAB               | pc-acoABCL   | acoR     | 88.11              | 70.73                 | 4.00                   | Huang et al. (1999), Ali et al. (2001), Delamarre and Batt (2006), Silbersack et al. (2006, 2006), Vivij et al. (2014), Hanko et al. (2020) |
| Trigonelline       | 5570       | ctgS1/ctgS2         | tgnAB        | nodD     | 137.14             | 314.5                 | 3.23                   | Schmidt et al. (1986), Ashihara (2008), Mizuno et al. (2014), Wang et al. (2017b), Perna et al. (2018) |
| Benzoate           | 242        | pal, 4cl, phoBCE    | benABCD      | benM     | 3700               | 121.11                | 3.42                   | Neidic et al. (1987), Otto et al. (2020)                                                   |
| cis,cis-Muconate   | 5280518    | pobA, aroY, catA    | catBC        | catR/benM| 142.11             | 132.5                 | 3.18                   | Parsek et al., 1992, Sengupta et al., 2015, Skjødt et al., 2016, Chai et al., 2020         |
| Luteolin           | 5280445    | tal, 4cl, chs, chi, fns, f3h | spnK         | nodD     | 286.24             | 2.32                  | Schmidt et al. (1986), Suominen et al. (2003), Peck et al. (2006), Marin et al. (2017), Bashyal et al. (2019), De Paepe et al. (2019) |
enough inducer to fully activate the sensors, leading to a poor dynamic range in their response. For this purpose, endogenous metabolic pathways may need to be tuned to increase precursor availability (Ni et al., 2015) or to avoid diffusor degradation (Adams and Jia, 2005). The development of robust diffusible signals and of bacterial strains that can reliably support this signalling is pivotal for the field of spatial pattern engineering and will potentially benefit synthetic biology in general.

Conclusion

The engineering of biological patterns could help untangle the complex mechanisms of development (Davies, 2017) and revolutionize organoid engineering and materials science. While many interesting patterns have already been built, the potential for innovation is still great. This is particularly true for multi-diffusor circuits, which could potentially show more diverse and complex spatiotemporal behaviours (Boehm et al., 2018; Barbier et al., 2020; Grant et al., 2020). We argue that the recent development of novel small-molecule diffusible signals will contribute to a development of spatial circuits, particularly of those with multiple diffusible components. We anticipate that recently discovered diffusive signals will enable synthetic biologists to engineer increasingly intricate, robust and tuneable spatial structures.

Conflict of interest

None declared.

References

Adams, M., and Jia, Z. (2005) Structural and biochemical analysis reveal Pirins to possess quercetinase activity. J Biol Chem 280: 28675–28682.
Ali, N.O., Bignon, J., Rapoport, G., and Debarbouille, M. (2001) Regulation of the acetoin catabolic pathway is controlled by sigma L in Bacillus subtilis. J Bacteriol 183: 2497–2504.
An, D.G., Yang, S.M., Kim, B.G., and Ahn, J.H. (2016) Biosynthesis of two quercetin O-diglycosides in Escherichia coli. J Ind Microbiol Biotechnol 43: 841–849.
Asakura, K., Konishi, R., Nakatani, T., Nakano, T., and Kamata, M. (2011) Turing pattern formation by the CIMA reaction in a chemical system consisting of quaternary alkyl ammonium cationic groups. J Phys Chem B 115: 3959–3963.
Ashihara, H. (2008) Trigonelline (N-methylnicotinic acid) biosynthesis and its biological role in plants. Nat Prod Commun 3: 1423–1428.
Barbier, I., Perez-Carrasco, R., and Schauerli, Y. (2020) Controlling spatiotemporal pattern formation in a concentration gradient with a synthetic toggle switch. Mol Syst Biol 16: e9361.
Bashyal, P., Parajuli, P., Pandey, R., and Sohng, J. (2019) Microbial biosynthesis of antibacterial chrysosierol in...
recombinant escherichia coli and bioactivity assessment. Catalysts 9: 112. http://dx.doi.org/10.3390/catal9020112
Basu, S., Gerchman, Y., Collins, C.H., Arnold, F.H., and Weiss, R. (2005) A synthetic multicellular system for programmed pattern formation. Nature 434: 1130–1134.
Blest, A.D. (1957) The function of eyespot patterns in the Lepidoptera. Behaviour 11: 209–258.
Boedicker, J., and Nealson, K. (2016) Microbial communication via quorum sensing. IEEE Trans Mol Biol Multi-Scale Commun 1: 310–320. http://dx.doi.org/10.1109/tmbmc.2016.2587629
Boehm, C.R., Grant, P.K., and Haseloff, J. (2018) Programmed hierarchical patterning of bacterial populations. Nat Commun 9: 776.
Boo, A., Amaro, R.L., and Stan, G.-B. (2021) Quorum sensing in synthetic biology: a review. Curr Opin Syst Biol 28: 100378.
Bottiglieri, M., and Keel, C. (2006) Characterization of PhlG, a hydrolase that specifically degrades the antifungal compound 2,4-diacetylphloroglucinol in the biocontrol agent Pseudomonas flourescens CHA0. Appl Environ Microbiol 72: 418–427.
Briscoe, J., and Small, S. (2015) Morphogen rules: design principles of gradient-mediated embryo patterning. Development 142: 3996–4009.
Butler, T., and Goldenfeld, N. (2011) Fluctuation-driven Turing patterns. Phys Rev E: Stat Nonlinear Soft Matter Phys 84: 11112.
Butzin, N.C., and Mather, W.H. (2018) Crosstalk between diverse synthetic protein degradation tags in Escherichia coli. ACS Synth Biol 7: 54–62.
Cachat, E., Liu, W., and Davies, J.A. (2017) Synthetic self-patterning and morphogenesis in mammalian cells: a proof-of-concept step towards synthetic tissue development. Eng Biol 1: 71–76.
Cao, Y., Ryser, M.D., Payne, S., Li, B., Rao, C.V., and You, L. (2016) Collective space-sensing coordinates pattern scaling in engineered bacteria. Cell 165: 620–630.
Carballido-Landeira, J., Vanag, V.K., and Epstein, I.R. (2010) Patterns in the Belousov-Zhabotinsky reaction in water-in-oil microemulsion induced by a temperature gradient. Phys Chem Phys 12: 3656.
Castets, V., Dulos, E., Boissonade, J., and De Kepper, P. (1990) Experimental evidence of a sustained standing Turing-type nonequilibrium chemical pattern. Phys Rev Lett 64: 2953–2956.
Ceroni, F., Algar, R., Stan, G.B., and Ellis, T. (2015) Quantifying cellular capacity identifies gene expression designs with reduced burden. Nat Methods 12: 415–418.
Choi, S., Lee, H.N., Park, E., Lee, S.J., and Kim, E.S. (2020) Recent advances in microbial production of cis,cis-muconic acid. Biromolecules 10: 1238.
Danino, T., Mondragón-Palomino, O., Tsimring, L., and Hasty, J. (2010) A synchronized quorum of genetic clocks. Nature 463: 326–330.
Davies, J. (2017) Using synthetic biology to explore principle of development. Development 144: 1146–1158.
Davies, J.A., and Glykofridis, F. (2020) Engineering pattern formation and morphogenesis. Bioch Soc Trans 48: 1177–1185.
involved in osmoprotection and ectoine catabolism. *J Bacteriol* **187**: 1293–1304.

Joshi, J.G., and Handler, P. (1960) Biosynthesis of trigonel-
line. *J Biol Chem* **235**: 2981–2983.

Karig, D., Martini, K.M., Lu, T., DeLateur, N.A., Goldenfeld, N., Weiss, R., et al. (2018) Stochastic Turing patterns in a synthetic bacterial population. *Proc Natl Acad Sci USA* **115**: 6572–6577.

Ko, Y.S., Kim, J.W., Chae, T.U., Song, C.W., and Lee, S.Y. (2020) A novel biosynthetic pathway for the production of acrylic acid through γ-alanine route in *Escherichia coli*. *ACS Synth Biol* **9**: 1150–1159.

Koga, S., and Kuramoto, Y. (1980) Localized patterns in reaction-diffusion systems. *Prog Theor Phys* **63**: 106–121.

Kong, W., Blanchard, A.E., Liao, C., and Lu, T. (2017) Engineering robust and tunable spatial structures with synthetic gene circuits. *Nucleic Acids Res* **45**: 1005–1014.

Kügler, P., Gaubitzer, E., and Müller, S. (2009) Parameter identification for chemical reaction systems using sparsity enforcing regularization: a case study for the chlorite-
iode reaction. *J Phys Chem A* **113**: 2775–2785.

Kumasov, O., Jablonski, L., Polanuyer, B., Dorrestein, P., Begley, T., and Osterman, A. (2003) Acetyl tropo
dehdration pathway in bacteria: novel kynurenine forma-
midase. *FEMS Microbiol Lett* **227**: 219–227.

Lee, H., Kim, B.G., Kim, M., and Ahn, J.H. (2015) Biosyn-
thesis of two flavones, apigenin and genkwanin, in *Escherichia coli*. *J Microbiol Biotechnol* **25**: 1442–1448.

Lengyel, I., and Epstein, I.R. (1992) A chemical approach to designing Turing patterns in reaction-diffusion systems. *Proc Natl Acad Sci USA* **89**: 3977–3979.

Lengyel, I.I., Kádár, S., and Epstein, I.R. (1993) Transient turing structures in a gradient-free closed system. *Science* **259**: 493–495.

Li, X., Rizik, L., Kravchik, V., Khoury, M., Korin, N., and Daniel, R. (2021) Synthetic neural-like computing in microbial consortia for pattern recognition. *Nat Commun* **12**: 3139.

Liao, L., Schaefer, A.L., Coutinho, B.G., Brown, P.J.B., and Peter Greenberg, E. (2018) An aryl-homoserine lactone quorum-sensing signal produced by a dimorphic prosthe-
dehesion. *Proc Natl Acad Sci USA* **115**: 7587–7592.

Liu, X., Liu, J., Lei, D., and Zhao, G.R. (2022) Modular metabo
lomic engineering for production of phloretic acid, phloretin and phlorizin in *Escherichia coli*. *Chem Eng Sci* **247**: 116931. http://dx.doi.org/10.1016/j.ces.2021.116931.

Luo, N., Wang, S., and You, L. (2019) Synthetic pattern for-
mation. *Biochemistry* **58**: 1478–1483.

Maini, P.K., Woolley, T.E., Baker, R.E., Gaffney, E.A., and Lee, S.S. (2012) Turing’s model for biological pattern for-
mation and the robustness problem. *Interface Focus* **2**: 487–496. http://dx.doi.org/10.1098/rsfs.2011.0113.

Marcon, L., Diego, X., Sharpe, J., and Müller, P. (2016) High-throughput mathematical analysis identifies turing networks for patterning with equally diffusing signals. *eLife* **5**: 14022.

Marin, L., Gutiérrez-del-Rio, I., Yagüe, P., Manteca, Á., Vil-
lar, C.J., and Lombó, F. (2017) De novo biosynthesis of apigenin, luteolin, and eriodictyol in the actinomycete *Streptomyces albus* and production improvement by feeding and spore conditioning. *Front Microbiol* **8**: 921.
Perchat, N., Saaidi, P.-L., Darii, E., Pellé, C., Petit, J.-L., Besnard-Gonnet, M., et al. (2018) Elucidation of the trigonelline degradation pathway reveals previously undescribed enzymes and metabolites. *Proc Natl Acad Sci USA* **115**: E4358–E4367.

Potvin-Trottier, L., Lord, N.D., Vinnicombe, G., and Paulson, J. (2016) Synchronous long-term oscillations in a synthetic gene circuit. *Nature* **538**: 514–517.

Purwins, H.G., Bodeker, H.U., and Amiranashvili, S. (2010) Dissipative solitons. *Adv Phys* **59**: 485–701.

Pušnik, Z., Mraz, M., Zimic, N., and Moskon, M. (2019) Computational analysis of viable parameter regions in models of synthetic biological systems. *J Biol Eng* **13**: 75.

Richter, A.A., Mais, C.-N., Czech, L., Geyer, K., Hoeppner, A., Smits, S.H.J., et al. (2014) A universal stochastic model of Turing network modulated by morphogen gradients. *Sci Rep* **3**: 566–570.

Richter, A.A., Smits, S.H.J., and Isalan, M. (2017) A three-step framework for programming pattern formation. *Curr Opin Chem Biol* **40**: 1–7.

Scholes, N.S., and Isalan, M. (2017) A three-step framework for programming pattern formation. *Curr Opin Chem Biol* **40**: 1–7.

Scholes, N.S., Schnoerr, D., Isalan, M., and Stumpf, M.P.H. (2019) A comprehensive network atlas reveals that turing patterns are common but not robust. *Cell Syst* **9**: 243–257.e4.

 Sekine, R., Shibata, T., and Ebisuya, M. (2018) Synthetic mammalian pattern formation driven by differential diffusivity of Nodal and Lefty. *Nat Commun* **9**: 1–11.

Sengupta, S., Jonnalagadda, S., Goonewardena, L., and Juturu, V. (2015) Metabolic engineering of a novel muconic acid biosynthesis pathway via 4-hydroxybenzoic acid in *Escherichia coli*. *Appl Environ Microbiol* **81**: 8037–8043.

Shats, I., Williams, J.G., Liu, J., Makarov, M.V., Wu, X., Lih, F.B., et al. (2020) Bacteria boost mammalian host NAD metabolism by engaging the deamidated biosynthesis pathway. *Cell Metab* **31**: 564–579.e7.

Sheth, R., Marcon, L., Bastida, M.F., Junco, M., Quintana, L., Dahn, R., et al. (2012) Hox genes regulate digit patterning by controlling the wavelength of a Turing-type mechanism. *Science* **338**: 1476–1480.

Siedler, S., Stahlhut, S.G., Malla, S., Maury, J.O., and Neves, A.R. (2014) Novel biosensors based on flavonoid-responsive transcriptional regulators introduced into *Escherichia coli*. *Metab Eng* **21**: 2–8.

Silbersack, J., Jürgen, B., Hecker, M., Schneider, B., Schmuck, R., and Schweder, T. (2006) An acetoin-regulated expression system of *Bacillus subtilis*. *Appl Microbiol Biotechnol* **73**: 895–903.

Silva, K.P.T., Chellamuthu, P., and Boedicker, J.Q. (2017) Quantifying the strength of quorum sensing crosstalk within microbial communities. *PLoS Comput Biol* **13**: e1005809.

Skjoedt, M.L., Snoek, T., Kildegaard, K.R., Arsovskia, D., Eichenberger, M., Goedecke, T.J., et al. (2016) Engineering prokaryotic transcriptional activators as metabolite biosensors in yeast. *Nat Chem Biol* **12**: 951–958.

Stahlhut, S.G., Siedler, S., Malla, S., Harrison, S.J., Maury, J., Neves, A.R., and Forster, J. (2015) Assembly of a novel biosynthetic pathway for production of the plant flavonoid fisetin in *Escherichia coli*. *Metab Eng* **31**: 84–93.

Stevens, M., Cuthill, I.C., Windsor, A.M.M., and Walker, H.J. (2006) Disruptive contrast in animal camouflage. *Proc R Soc B* Sci **273**: 2433–2438.

Strauss, S., Lempe, J., Prusinkiewicz, P., Tsiantis, M., and Smith, R.S. (2020) Phyllotaxis: is the golden angle optimal for light capture? *New Phytol* **225**: 499–510.

Suominen, L., Luukkainen, R., Roos, C., and Lindström, K. (2003) Activation of the nodA promoter by the nodD genes of *Rhizobium galegae* induced by synthetic flavonoids or *Galega orientalis* root exudate. *FEBS Microbiol Lett* **219**: 225–232.

Szalai, I., and De Kepper, P. (2008) Pattern formation in the ferrocyanide-iodate-sulfite reaction: the control of space scale separation. *Chaos* **18**: 26105.

Tamsir, A., Tabor, J.J., and Voigt, C.A. (2011) Robust multicellular computing using genetically encoded NOR gates and chemical "wires". *Nature* **469**: 212–215.

Tang, C.D., Shi, H.L., Xu, J.H., Liu, F., Jiao, Z.J., Ding, P.J., et al. (2018) Biosynthesis of phenylglyoxylic acid by *LhDMDH*, a novel d -Mandela dehydrogenase with high catalytic activity. *J Agric Food Chem* **66**: 2805–2811.

Tsek, S.J., Smith, C.L., Lopez, B., Mani, A., Connot, C., Livingstone, X., and Haynes, K.A. (2019) Engineered orthogonal quorum sensing systems for synthetic gene regulation in *Escherichia coli*. *Front Bioeng Biotechnol* **7**: 80.

Tica, J., Zhu, T., and Isalan, M. (2020) Dynamical model fitting to a synthetic positive feedback circuit in *E. coli*. *Eng Biol* **4**: 25–31.

Tordoff, J., Krajcí, M., Walczak, N., Lima, M., Beal, J., Shvartsman, S., and Weiss, R. (2021) Incomplete cell sorting creates engineerable structures with long-term stability. *Cell Rep Phys Sci* **2**: 100305. http://dx.doi.org/10.1016/j.xcrp.2020.100305

Turányi, T. (1994) Parameterization of reaction mechanisms using orthonormal polynomials. *Comput Chem* **18**: 45–54.

Turing, A.M. (1952) The chemical basis of morphogenesis. *Philos Trans R Soc Lond B Biol Sci* **237**: 37–72.

Vargas, P., Felipe, A., Michán, C., and Gallegos, M.T. (2011) Induction of Pseudomonas syringae pv. tomato
DC3000 mexAB-oprM multidrug efflux pump by flavonoids is mediated by the repressor PmeR. *Mol Plant-microbe Interact* **24**: 1207–1219.

Vivij, B., Moons, P., Aertsen, A., and Michiels, C.W. (2014) Acetoin synthesis acquisition favors *Escherichia coli* growth at low pH. *Appl Environ Microbiol* **80**: 6054–6061.

Wang, J., Shen, X., Jain, R., Wang, J., Yuan, Q., and Yan, Y. (2017a) Establishing a novel biosynthetic pathway for the production of 3,4-dihydroxybutyric acid from xylose in *Escherichia coli*. *Metab Eng* **41**: 39–45.

Wang, X., Zhou, Y.J., Wang, L., Liu, W., Liu, Y., Peng, C., and Zhao, Z.K. (2017b) Engineering *Escherichia coli* nicotinic acid mononucleotide adenyllytransferase for fully active amidated NAD biosynthesis. *Appl Environ Microbiol* **83**: e00692-17.

Wu, W., Liu, F., and Singh, S. (2018) Toward engineering *E. coli* with an autoregulatory system for lignin valorization. *Proc Natl Acad Sci USA* **115**: 2970–2975.

Yeoh, J.W., Ng, K.B.I., Teh, A.Y., Zhang, J.Y., Chee, W.K.D., and Poh, C.L. (2019) An Automated Biomodel Selection System (BMSS) for gene circuit designs. *ACS Synth Biol* **8**: 1484–1497.

Zhang, H., Wang, Y., Wu, J., Skalina, K., and Pfeifer, B.A. (2010) Complete biosynthesis of erythromycin A and designed analogs using *E. coli* as a heterologous host. *Chem Biol* **17**: 1232–1240.

Zheng, M.M., Shao, B., and Ouyang, Q. (2016) Identifying network topologies that can generate turing pattern. *J Theor Biol* **408**: 88–96.