Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Microbiology

Engineering synthetic spatial patterns in microbial populations and communities

Içvara Barbier*, Hadiastri Kusumawardhani# and Yolanda Schaerli$

Spatial pattern formation is an important feature of almost all biological systems. Thanks to the advances in synthetic biology, we can engineer microbial populations and communities to display sophisticated spatial patterns. This bottom-up approach can be used to elucidate the general principles underlying pattern formation. Moreover, it is of interest for a plethora of applications, from the production of novel living materials to medical diagnostics. In this short review, we comment on the recent experimental advances in engineering the spatial patterns formed by microbes. We classify the synthetic patterns based on the input signals provided and the biological processes deployed. We highlight some applications of microbial pattern formation and discuss the challenges and potential future directions.

Address
Department of Fundamental Microbiology, University of Lausanne, Switzerland

Corresponding author: Yolanda Schaerli (yolanda.schaerli@unil.ch)
* ORCID: 0000-0001-6814-2871
# ORCID:0000-0003-2755-5017
$ ORCID: 0000-0002-9083-7343

Current Opinion in Microbiology 2022, **67**:102149

This review comes from a themed issue on **Systems and Synthetic Biology**

Edited by **Rainer Breitling** and **Eriko Takano**

https://doi.org/10.1016/j.mib.2022.102149

Introduction

Spatial patterns are omnipresent across all scales of living entities, including patterns of proteins inside cells [1], of gene expression across embryonic tissues [2], and of colors in furs or skins [3]. Populations and communities of microorganisms also organize in spatial patterns. Indeed, spatial organization of microbial species shapes their interactions and the function of diverse microbiotas, for example in the gut, in wastewater treatment and in bioproduction [4–6].

The classical top-down approach to study natural patterns can be complemented with a bottom-up approach that leverages the design–build–test–learn cycle — the paradigm of synthetic biology [7,8]. This allows researchers to construct simplified patterning systems that are amenable to study or to modify naturally existing patterns. It facilitates the discovery and testing of general principles underlying complex patterning processes [9]. In addition, synthetic biology enables researchers to implement spatiotemporal patterns for novel applications such as engineered living materials [10,11].

In this short review, we focus on the recent experimental works that use synthetic biology to produce spatial patterns in microbial populations and communities. First, we introduce the different inputs commonly used to engineer synthetic patterns (Figure 1a). Next, we explore the different processes to interpret these inputs and convert them into spatial patterns (Figure 1b) and highlight some applications of such synthetic patterns. Finally, we discuss the remaining challenges in engineering pattern formation.

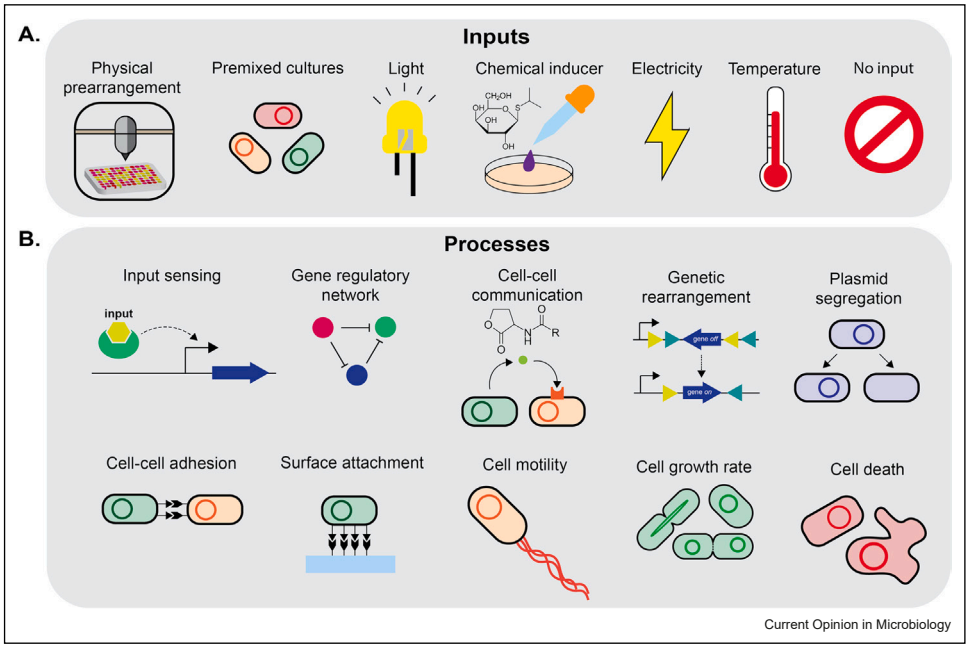
Inputs for pattern formation

Patterns can either be completely self-organizing or require some external input information. Several modes to provide such an initial input have been applied in recent years (Figure 1a). The input can be very rich in information, such as positioning the cells already in a spatial arrangement or providing a detailed motif with light, or it can also be of low information content such as the concentration gradient of a chemical inducer. Here, we briefly describe the different inputs used in synthetic biology to engineer spatial patterns.

Physical prearrangement

Prepositioning of cells in a spatial arrangement can be low-tech, such as simply plating the sender cells next to the receiver cells [12], or more sophisticated, such as using liquid-handling robots [13], meniscus-driven fluidic systems [14], or 3D printing [15–17]. The latter allows not only 2D patterning, but also the generation of 3D structures, thanks to ink containing genetically engineered microbes [15–17].

Figure 1



Schematic representations of the different inputs and processes to engineer spatial patterns in microbial populations. Inputs **(a)** and processes **(b)** that have been applied for engineering patterns in microbial populations. Different inputs and processes may be combined to generate complex spatial patterns.

Premixed cultures

Having a single bacterial population carrying all the same synthetic circuit often limits the complexity of the patterns that can be achieved because of the metabolic load, context-dependent effects, and incompatible molecular parts. Mixing multiple interacting strains resulting in a small microbial consortium can help address this issue through division of labor [18]. For example, mixing different cell types harboring different adhesion interactions leads to the formation of patterned cell clusters without the need to engineer complex cell differentiation [19,20]. Similarly, Curatolo and colleagues engineered two populations of motile *Escherichia coli (E. coli)* cells that can communicate with each other to reciprocally control their motilities, resulting in ring patterns [21].

Light

Using light to control the activity of cells, that is, optogenetics, is becoming increasingly important in the field of patterning. It enables a high level of precision and flexibility in both space and time [22–28]. Light can provide different amounts of information content. While most studies use light to provide a high level of initial spatial information, it can also be used to provide gradients as initial input. This kind of input could be ideal to study the patterning driven by diffusible signaling molecules, since different diffusion rates, half-lives, and dynamic changes can more easily be tested than with chemical molecules [29].

Chemical inducers

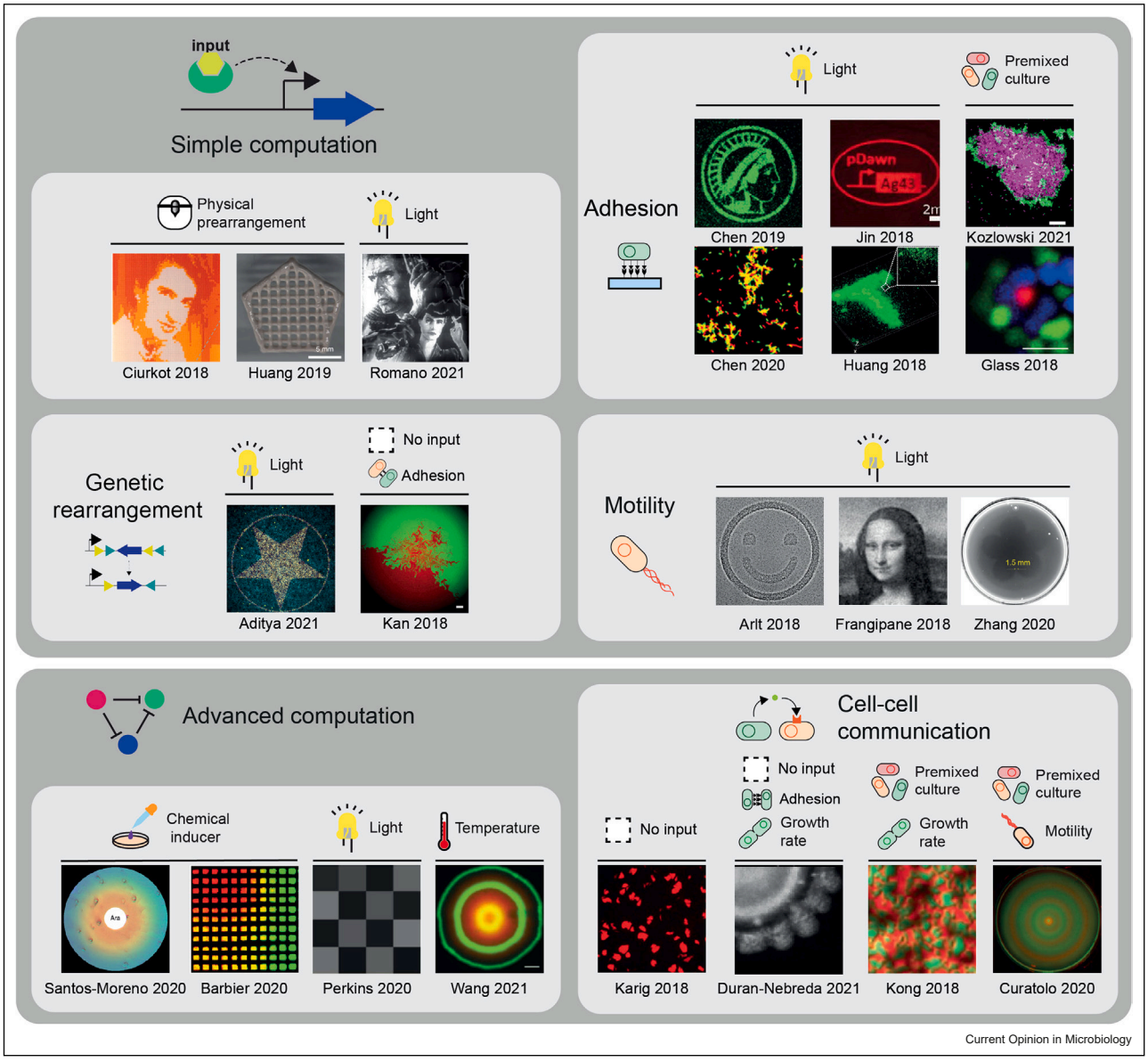
Chemical molecules are very commonly used as input for engineered patterns. Examples include inducers from classical gene expression systems such as arabinose, (isopropyl ß-D-1-thiogalactopyranoside), and (anhydrotetracycline), but also molecules from quorum-sensing systems and other small molecules derived from intracellular metabolites. These molecules can be added to the media [12,30,31] or produced by the bacteria [32–34], thus creating a self-regulating system. If they are deposited or produced locally, the forming spatial gradients can provide positional information, which is interpreted by the cells to give rise to patterns [30,31,35].

Compared to optogenetics and 3D printing methods, the use of chemical molecules has the disadvantage of being less precise and adjustable. For example, it is nontrivial to control their diffusion rates or to remove them on demand. On the other hand, this method requires less human intervention to achieve patterning, closely emulates natural patterning systems using diffusible molecules, and can lead to truly self-organized patterns.

Others

We summarized above the main inputs used for patterning in the last years. However, this list is not exhaustive. For example, electricity can also be coupled with cellular redox reactions to induce cell–cell signaling [36] or temperature

Figure 2



Examples of engineered spatial patterns in synthetic microbial populations and communities. The combinations of input signals and processes used to produce the spatial patterns are indicated with the schemes of Figure 1. Pictures reproduced with permission from the cited publications.

can be used to control gene expression and form patterns [37]. Cell morphology also plays an important role in the spatial patterning of microbial communities [19,38]. Other spatial organizations can be developed in the absence of external inputs, leading to genuine self-forming patterns [32,33,39,40]. In these studies, the differentiation between cells is initiated either by molecular noise [32,33] or by unstable initial states [39].

Processes for pattern formation

After sensing the input signal, microbes need to process the received information in order to produce a spatial pattern. Here, we briefly discuss the different ways of information processing that have been used in recent studies (Figure 1b). However, engineered patterns with microorganisms rarely use only one process, but mostly use a combination of different mechanisms (Figure 2).

Simple computation

The most straightforward process is to use sensor elements to translate the input into a biological output, such as the expression of a fluorescent reporter. Although easy to implement, a simple computation requires the input to contain already all of the patterning information. This is exemplified in a study carried out by Romano and colleagues, in which they engineered the transcription factor AraC to detect blue light instead of L-arabinose. This modification allowed a precise reconstruction of complex light pictures by a lawn of bacteria expressing green fluorescent protein [27]. Sensing an external input is also a step used in combination with most of the other mechanisms described below.

Advanced computation by gene regulatory networks

The computation performed by gene-regulatory networks enables transforming a simple input into a more complex output. The most iconic example is the formation of a stripe or 'French flag' pattern by feed-forward motifs starting from an input present as concentration gradient [35,41]. Another example of a patterning motif is the toggle switch. This gene-regulatory network is composed of two nodes mutually repressing each other and allows the transformation of a continuous chemical gradient into a binary response with a sharp boundary [30,31].

Temporal patterns, such as oscillations, can also lead to periodic spatial motifs. For instance, *E. coli* cells carrying the 'repressilator', a network of three transcription factors repressing each other in a closed loop producing temporal oscillations, show a ring pattern when grown in solid culture [42,43].

In 'cybergenetics', the computation is split between the cells and a computer, which takes the real-time measured expression levels as input and produces in turn light signals that dynamically control the behavior of the cells. Using this cell-in-the-loop approach, Perkins and colleagues engineered a checkerboard pattern governed by lateral inhibition in a population of yeast cells [26].

Genetic rearrangement

Cells can also be engineered to change their genetic architecture during the patterning process, leading to stable differentiated cell types. For example, Aditya and colleagues used blue light to induce the excision of a gene coding for a fluorescent marker with the Cre/lox system. In this way, spatial patterns composed of two differentiated yeast strains encoding and expressing distinct fluorescence reporters were formed in response to the projected light [22]. Plasmid segregation in *E. coli* was also used to break the symmetry and produce spatial patterns in growing colonies [39,44].

Motility

Most synthetic patterns are visualized by reporter genes. However, spatial motifs can also be formed by modulating the cell density. One way to influence the cell density is to spatially control the cell motility. Several studies controlling the motility of bacteria by light have reproduced images with cells. Different ways of interfacing light with cell motility have been implemented, such as expressing proteorhodopsin, a light-driven proton pump powering the rotation of the flagellar motor [23,45], or modulating the expression of CheZ, a phosphatase involved in controlling the flagella rotation in *E. coli*, with a light-inducible promoter [28]. One advantage of using light to control motility is that the images can be easily and rapidly reconfigured [45].

Adhesion

Cell adhesion to surfaces or to other cells can also be used to control the position or arrangement of cells. Light has been used to promote cell surface adhesion, by modifying either the adhesion properties of the bacteria [25,46] or those of the surface [24]. Several natural and synthetic adhesins (including nanobodies/antigens and SpyTag/SpyCatcher) have been used to promote cell–-cell adhesion. The availability of multiple orthogonal adhesion systems with different strengths enabled the programming of patterns based on phase separation, differential adhesion, and sequential layering in liquid microbial cultures [19,20]. Cell-to-cell adhesion can also be controlled by light and was used to produce reversible cellular aggregation patterns [47]. Kan and colleagues combined intercellular adhesion with the previously described plasmid segregation system and showed that adhesion promoted elongation of naturally occurring fractal patterns at the boundary of cell lineages in bacterial colonies [44].

Cell–cell communication

Engineered cell–cell communication is an important element of many patterning endeavors. Most of the times, the communication is based on small diffusible molecules (mainly sourced from quorum-sensing systems), but it can also take other forms, such as light [48] or phages [49]. Combining the cell's production of small diffusible molecules with their sensing and ability to control gene expression allows the generation of patterns based on reaction-diffusion mechanisms [50]. Karig and colleagues engineered *E. coli* to produce and react to two quorum-sensing molecules: one activated the synthesis of both molecules, while the other inhibited the synthesis of both. When grown as bacterial lawn, these *E. coli* strains displayed irregular spot patterns in the expression of fluorescent reporters that are consistent with stochastic Turing theory [33].

Cell growth and cell death

Cell growth and cell death also influence cell densities and arrangements. One way of controlling them is with ecological interactions between species in microbial communities, such as predation, neutralism, cooperation, or competition [51]. Duran-Nebreda and colleagues combined growth inhibition, cell elongation, adhesion, and cell–cell communication via diffusible quorum-sensing molecules to engineer periodic patterns in forming bacterial colonies [32]. A Type VI secretion system (T6SS)-mediated killing mechanism was shown to drive phase separation in dense bacterial populations [52]. In combination with synthetic cell–cell adhesion, T6SS-mediated killing was also used to eliminate specified target cells from a microbial community [53].

Applications

Some of the above-described studies contributed to improving our understanding of the mechanisms involved in pattern formation that are also at play in natural prokaryotic and/or eukaryotic patterning systems. Synthetic biology allows us to construct simplified versions of complex natural patterning systems that are amenable to study. We can thus focus on the elements of interest, while avoiding confounding factors present in natural systems. It enables us to test and discover the general principles underlying complex patterning processes [9,54,55]. Other studies focused more on the engineering aspects of microbial spatial patterns, which have various potential applications ranging from providing tools for biomedical research [56], enhancing bioproduction by division of labor [18,57], performing complex, distributed biocomputation [58,59], and producing patterned engineered living materials [10,11]. Here, we highlight a few of them.

The work of Riglar and colleagues provides an example of how spatiotemporal pattern-forming bacteria can be used for biomedical research [56]. They used *E. coli* cells with engineered temporal oscillations as a marker of bacterial growth dynamics at the single-cell level in response to inflammation in a murine gut. After transitioning through the gut, cells recovered from the feces were plated and colonies with fluorescent ring patterns were formed. The positions of the fluorescent rings in a colony were indicative of the phase of the bacterium that seeded the colony, and thus allowed to infer the number of divisions that occurred in the gut. The analysis of multiple colonies also enabled to assess the growth variability among cells.

Natural biomaterials created by living systems exhibit self-organization into patterns, and have the ability to sense and respond to their environmental conditions. The field of engineered living materials (ELM) aims to produce novel materials using genetically encoded functionalities. Several examples of spatiotemporal patterning in ELMs have been reported in recent years. Wang and colleagues produced patterned living mineralized composites by combining light-inducible bacterial biofilm formation and biomimetic hydroxyapatite mineralization [60]. Curli fibers are the main proteinaceous extracellular component of *E. coli* biofilms and can be assembled into biodegradable bioplastic with self-repair ability [61]. A combination of genetic regulation of curli formation and spatial control by 3D printing successfully created patterned materials with potential applications in drug delivery, bioremediation, water filtration, metal ion sequestration, and civil engineering [17,62,63]. In bacterial cellulose-based ELMs, the formation of spatial patterns has been controlled by optogenetics and chemical inducers [64,65]. Bacterial cellulose can be functionalized with enzymes to generate catalytic materials [64] or with growth factors for applications in tissue engineering [65]. Furthermore, the properties of ELMs, such as the ability to self-repair, viscoelasticity, and mechanical rigidity, can be further improved by programmable cell–cell adhesion [66].

Conclusions and outlook

The use of synthetic biology to engineer the spatial patterns in microbial populations and communities is a very active area of research. In this short review, we summarized the main inputs and processes that have recently been employed to generate synthetic patterns, and we also highlighted some applications of such patterns. Even though we focused on the experimental part, mathematical modeling also plays a crucial role in engineering spatial patterns [67].

Despite impressive progress, there are still challenges that remain to be addressed. For example, it would be desirable to be able to produce more complex patterns without relying on high-content inputs like light or 3D printing. We have several suggestions to achieve more sophisticated patterns without increasing the input complexity. One of them is to produce patterns relying on reaction-diffusion mechanisms, such as Turing patterns. They can generate complex periodic patterns that are completely self-organizing. Despite considerable efforts, synthetic Turing patterns have not been achieved yet. However, recent experimental [33,68] as well as theoretical progress [69] makes us hopeful that this achievement will soon be accomplished. Another promising approach would be to develop cell–cell contact-dependent signaling, which is an important process in natural pattern-forming systems. This form of communication is for example essential for lateral inhibition

[70], where a cell with a particular fate prevents its immediate neighboring cells from adopting the same fate, resulting in checkerboard patterns. While signaling based on synthetic Notch [71] is a powerful contact-dependent cell–cell communication platform in mammalian synthetic biology [72,73], to the best of our knowledge, a bacterial analog that delivers something else than toxins [52,53] has not yet been exploited for synthetic pattern formation.

Most of the literature covered here has been performed with the model organisms *E. coli* and *Saccharomyces cerevisiae*. They are the most studied and well-understood microbes, and a plethora of tools to genetically modify them have been developed. However, the field would certainly benefit from expanding also to other organisms. For example, microbes already displaying a particular spatiotemporal pattern could be used as the basis to modify the pattern as desired. Establishing patterning tools in different organisms will help consolidate the knowledge gained so far, and real-world applications will benefit from a larger diversity of available 'chassis' that might potentially be more suitable [74].

Another limitation of synthetic pattern formation is that so far most of the studies focused on the patterns in 2D, but expanding to 3D would certainly be desirable. The field of ELM started to explore the third dimension using 3D printing, molding, and mineralization, and we expect to see further progress in near future [15,60,61].

So far, most microbial patterning projects have only combined few patterning processes. Combining multiple patterning mechanisms in a hierarchical manner, such as phase separation based on adhesion of two populations, followed by cell–cell communication between them and information processing by gene-regulatory networks, will open the doors to produce more complex spatial patterns, similar to what has already been achieved with mammalian cells [72,73]. To engineer such multistep patterning programs, the field will benefit from recent and future advances in synthetic biology, such as the use of artificial intelligence and whole-cell simulations [75]. In the future, machine learning may be implemented to design intricate gene-regulatory networks for a desired output [76].

We are confident that if the field addresses the challenges described here, the research on engineering synthetic spatial patterns in microbial populations and communities will continue to thrive and to improve our understanding of natural pattern formation, as well as lead to numerous real-world applications.

Conflict of interest statement

Nothing declared.

Acknowledgements

The work was supported by the Swiss National Science Foundation through project grants [31003A_175608 and 310030_200532] and the NCCR Microbiomes, a National Centre of Competence in Research, funded by the Swiss National Science Foundation (grant number 180575).

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Denk J, Kretschmer S, Halatek J, Hartl C, Schwille P, Frey E: **MinE conformational switching confers robustness on self-organized Min protein patterns**. *Proc Natl Acad Sci* 2018, **115**:4553-4558.
2. Jaeger J: **The gap gene network**. *Cell Mol Life Sci* 2011, **68**:243-274.
3. Kondo S, Watanabe M, Miyazawa S: **Studies of turing pattern formation in zebrafish skin**. *Philos Trans R Soc A Math Phys Eng Sci* 2021, **379**:20200274.
4. Ben Said S, Tecon R, Borer B, Or D: **The engineering of spatially linked microbial consortia – potential and perspectives**. *Curr Opin Biotechnol* 2020, **62**:137-145.
5. Liu W, Tokuyasu TA, Fu X, Liu C: **The spatial organization of microbial communities during range expansion**. *Curr Opin Microbiol* 2021, **63**:109-116.
6. Tropini C, Earle KA, Huang KC, Sonnenburg JL: **The gut microbiome: connecting spatial organization to function**. *Cell Host Microbe* 2017, **21**:433-442.
7. Bashor CJ, Collins JJ: **Understanding biological regulation through synthetic biology**. *Annu Rev Biophys* 2018, **47**:399-423.
8. Wang L-Z, Wu F, Flores K, Lai Y-C, Wang X: **Build to understand: synthetic approaches to biology**. *Integr Biol UK* 2015, **8**:394-408.
9. Santos-Moreno J, Schaerli Y: **Using synthetic biology to engineer spatial patterns**. *Adv Biosyst* 2019, **3**:1800280.
10. Burgos-Morales O, Gueye M, Lacombe L, Nowak C, Schmachtenberg R, Hörner M, Jerez-Longres C, Mohsenin H, Wagner HJ, Weber W: **Synthetic biology as driver for the biologization of materials sciences**. *Mater Today Bio* 2021, **11**:100115.
11. Gilbert C, Ellis T: **Biological engineered living materials: growing functional materials with genetically programmable properties**. *ACS Synth Biol* 2019, **8**:1-15.
12. Boehm CR, Grant PK, Haseloff J: **Programmed hierarchical patterning of bacterial populations**. *Nat Commun* 2018, **9**:776.
13. Ciurkot K, Vonk B, Gorochowski TE, Roubos JA, Verwaal R: **CRISPR/Cas12a multiplex genome editing of saccharomyces cerevisiae and the creation of yeast pixel art**. *J Vis Exp* 2019, **28**:147.
14. Kantsler V, Ontañón-McDonald E, Kuey C, Ghanshyam MJ, Roffin MC, Asally M: **Pattern engineering of living bacterial colonies using meniscus-driven fluidic channels**. *ACS Synth Biol* 2020, **9**:1277-1283.
15. Duraj-Thatte AM, Manjula-Basavanna A, Rutledge J, Xia J, Hassan S, Sourlis A, Rubio AG, Lesha A, Zenkl M, Kan A, *et al.*: **Programmable microbial ink for 3D printing of living materials produced from genetically engineered protein nanofibers**. *Nat Commun* 2021, **12**:6600.
16. Huang J, Liu S, Zhang C, Wang X, Pu J, Ba F, Xue S, Ye H, Zhao T, Li K, *et al.*: **Programmable and printable Bacillus subtilis biofilms as engineered living materials**. *Nat Chem Biol* 2019, **15**:34-41.
17. Schmieden DT, Basalo Vázquez SJ, Sangüesa H, van der Does M, Idema T, Meyer AS: **Printing of patterned, engineered E. coli**

biofilms with a low-cost 3D printer. *ACS Synth Biol* 2018, **7**:1328-1337.

18. Sgobba E, Wendisch VF: **Synthetic microbial consortia for small molecule production**. *Curr Opin Biotechnol* 2020, **62**:72-79.

19. • Glass DS, Riedel-Kruse IH: **A synthetic bacterial cell-cell adhesion toolbox for programming multicellular morphologies and patterns**. *Cell* 2018, **174**:649-658 e616.
This paper describes a genetically encoded synthetic platform for modular cell – cell adhesion in *E. coli*, which allows multicellular self-assembly. This tool can be implemented to engineer spatial patterns using homophilic and heterophilic interactions, lattice-like self-assembly, phase separation, differential adhesion, and sequential layering.

20. Kozlowski MT, Silverman BR, Johnstone CP, Tirrell DA: **Genetically programmable microbial assembly**. *ACS Synth Biol* 2021, **10**:1351-1359.

21. • Curatolo AI, Zhou N, Zhao Y, Liu C, Daerr A, Tailleur J, Huang J: **Cooperative pattern formation in multi-component bacterial systems through reciprocal motility regulation**. *Nat Phys* 2020, **16**:1152-1157.
This paper describes the self-organization of two cell populations by reciprocal control of their motilities. Without the need of any positional information as input, the two initial mixed populations of engineered *E. coli* cells segregate, leading to population oscillations and ring patterns.

22. • Aditya C, Bertaux F, Batt G, Ruess J: **A light tunable differentiation system for the creation and control of consortia in yeast**. *Nat Commun* 2021, **12**:5829.
This paper describes a light-driven differentiation system in *S. cerevisiae* that is capable of generating a microbial consortium consisting of functionally different subpopulations with user-defined composition in space and in time from a single starting strain.

23. Arlt J, Martinez VA, Dawson A, Pilizota T, Poon WCK: **Painting with light-powered bacteria**. *Nat Commun* 2018, **9**:768.

24. Chen F, Ricken J, Xu D, Wegner SV: **Bacterial photolithography: patterning escherichia coli biofilms with high spatial control using photocleavable adhesion molecules**. *Adv Biosyst* 2019, **3**:1800269.

25. Huang Y, Xia A, Yang G, Jin F: **Bioprinting living biofilms through optogenetic manipulation**. *ACS Synth Biol* 2018, **7**:1195-1200.

26. Perkins ML, Benzinger D, Arcak M, Khammash M: **Cell-in-the-loop pattern formation with optogenetically emulated cell-to-cell signaling**. *Nat Commun* 2020, **11**:1355.

27. • Romano E, Baumschlager A, Akmeriç EB, Palanisamy N, Houmani M, Schmidt G, Öztürk MA, Ernst L, Khammash M, Di, Ventura B: **Engineering AraC to make it responsive to light instead of arabinose**. *Nat Chem Biol* 2021, **17**:817-827.
This paper describes an engineered blue light-inducible AraC dimer in *E. coli* to achieve spatiotemporal control of gene expression.

28. Zhang J, Luo Y, Poh CL: **Blue light-directed cell migration, aggregation, and patterning**. *J Mol Biol* 2020, **432**:3137-3148.

29. Baumschlager A, Khammash M: **Synthetic biological approaches for optogenetics and tools for transcriptional light-control in bacteria**. *Adv Biol* 2021, **5**:2000256.

30. Grant PK, Szep G, Patange O, Halatek J, Coppard V, Csikasz-Nagy A, Haseloff J, Locke JCW, Dalchau N, Phillips A: **Interpretation of morphogen gradients by a synthetic bistable circuit**. *Nat Commun* 2020, **11**:5545.

31. •• Barbier I, Perez-Carrasco R, Schaerli Y: **Controlling spatiotemporal pattern formation in a concentration gradient with a synthetic toggle switch**. *Mol Syst Biol* 2020, **16**:e9361.
In this paper, the authors present a synthetic toggle switch controllable by diffusible molecules in *E. coli*. By combining quantitative measurements with a mathematical model, the authors demonstrate how the hysteresis, position, timing, and precision of the boundary can be controlled.

32. Duran-Nebreda S, Pla J, Vidiella B, Pinero J, Conde-Pueyo N, Sole R: **Synthetic lateral inhibition in periodic pattern forming microbial colonies**. *ACS Synth Biol* 2021, **10**:277-285.

33. Karig D, Martini KM, Lu T, DeLateur NA, Goldenfeld N, Weiss R: **Stochastic Turing patterns in a synthetic bacterial population**. *Proc Natl Acad Sci* 2018, **115**:6572-6577.

34. Du P, Zhao H, Zhang H, Wang R, Huang J, Tian Y, Luo X, Luo X, Wang M, Xiang Y, *et al*.: **De novo design of an intercellular signaling toolbox for multi-channel cell-cell communication and biological computation**. *Nat Commun* 2020, **11**:4226.

35. Santos-Moreno J, Tasiudi E, Stelling J, Schaerli Y: **Multistable and dynamic CRISPRi-based synthetic circuits**. *Nat Commun* 2020, **11**:2746.

36. Terrell JL, Tschirhart T, Jahnke JP, Stephens K, Liu Y, Dong H, Hurley MM, Pozo M, McKay R, Tsao CY, *et al*.: **Bioelectronic control of a microbial community using surface-assembled electrogenetic cells to route signals**. *Nat Nanotechnol* 2021, **16**:688-697.

37. Wang X, Han JN, Zhang X, Ma YY, Lin Y, Wang H, Li DJ, Zheng TR, Wu FQ, Ye JW, *et al*.: **Reversible thermal regulation for bifunctional dynamic control of gene expression in Escherichia coli**. *Nat Commun* 2021, **12**:1411.

38. Smith WPJ, Davit Y, Osborne JM, Kim W, Foster KR, Pitt-Francis JM: **Cell morphology drives spatial patterning in microbial communities**. *Proc Natl Acad Sci* 2017, **114**:E280-E286.

39. Nunez IN, Matute TF, Del Valle ID, Kan A, Choksi A, Endy D, Haseloff J, Rudge TJ, Federici F: **Artificial symmetry-breaking for morphogenetic engineering bacterial colonies**. *ACS Synth Biol* 2017, **6**:256-265.

40. Payne S, Li B, Cao Y, Schaeffer D, Ryser MD, You L: **Temporal control of self-organized pattern formation without morphogen gradients in bacteria**. *Mol Syst Biol* 2013, **9**:697.

41. Basu S, Gerchman Y, Collins CH, Arnold FH, Weiss R: **A synthetic multicellular system for programmed pattern formation**. *Nature* 2005, **434**:1130-1134.

42. Yáñez Feliú G, Vidal G, Muñoz Silva M, Rudge TJ: **Novel tunable spatio-temporal patterns from a simple genetic oscillator circuit**. *Front Bioeng Biotechnol* 2020, **8**:893.

43. Potvin-Trottier L, Lord ND, Vinnicombe G, Paulsson J: **Synchronous long-term oscillations in a synthetic gene circuit**. *Nature* 2016, **538**:514-517.

44. Kan A, Del Valle I, Rudge T, Federici F, Haseloff J: **Intercellular adhesion promotes clonal mixing in growing bacterial populations**. *J R Soc Interface* 2018, **15**:20180406.

45. •• Frangipane G, Dell'Arciprete D, Petracchini S, Maggi C, Saglimbeni F, Bianchi S, Vizsnyiczai G, Bernardini ML, Di, Leonardo R: **Dynamic density shaping of photokinetic E. coli**. *Elife* 2018, **7**:e36608.
In this paper, the authors demonstrate spatiotemporal control of cell density to create complex and dynamic density patterns by using a digital light projector that regulates photokinetic movements

46. Jin X, Riedel-Kruse IH: **Biofilm Lithography enables high-resolution cell patterning via optogenetic adhesin expression**. *Proc Natl Acad Sci* 2018, **115**:3698-3703.

47. Chen F, Wegner SV: **Blue-light-switchable bacterial cell–cell adhesions enable the control of multicellular bacterial communities**. *ACS Synth Biol* 2020, **9**:1169-1180.

48. Timsit Y, Lescot M, Valiadi M, Not F: **Bioluminescence and photoreception in unicellular organisms: light-signalling in a bio-communication perspective**. *Int J Mol Sci* 2021, **22**:11311.

49. Ortiz ME, Endy D: **Engineered cell-cell communication via DNA messaging**. *J Biol Eng* 2012, **6**:16.

50. Kondo S, Miura T: **Reaction-diffusion model as a framework for understanding biological pattern formation**. *Science* 2010, **329**:1616-1620.

51. Kong W, Meldgin DR, Collins JJ, Lu T: **Designing microbial consortia with defined social interactions**. *Nat Chem Biol* 2018, **14**:821-829.

52. McNally L, Bernardy E, Thomas J, Kalziqi A, Pentz J, Brown SP, Hammer BK, Yunker PJ, Ratcliff WC: **Killing by Type VI secretion drives genetic phase separation and correlates with increased cooperation**. *Nat Commun* 2017, **8**:14371.

53. Ting SY, Martínez-García E, Huang S, Bertolli SK, Kelly KA, Cutler KJ, Su ED, Zhi H, Tang Q, Radey MC, *et al.*: **Targeted depletion of bacteria from mixed populations by programmable adhesion with antagonistic competitor cells**. *Cell Host Microbe* 2020, **28**:313-321 e316.

54. Ebrahimkhani MR, Ebisuya M: **Synthetic developmental biology: build and control multicellular systems**. *Curr Opin Chem Biol* 2019, **52**:9-15.

55. Luo N, Wang S, You L: **Synthetic pattern formation**. *Biochemistry* 2019, **58**:1478-1483.

56. •• Riglar DT, Richmond DL, Potvin-Trottier L, Verdegaal AA, Naydich AD, Bakshi S, Leoncini E, Lyon LG, Paulsson J, Silver PA: **Bacterial variability in the mammalian gut captured by a single-cell synthetic oscillator**. *Nat Commun* 2019, **10**:4665.

In this paper, the authors use a synthetic oscillator in *E. coli* cells to keep time in the mouse gut over periods of days. This provides information regarding bacterial growth and dynamics in response to inflammation and other variations in the gut microbiota.

57. Volke DC, Nikel PI: **Getting bacteria in shape: synthetic morphology approaches for the design of efficient microbial cell factories**. *Adv Biosyst* 2018, **2**:1800111.

58. Goñi-Moreno A, Nikel PI: **High-performance biocomputing in synthetic biology–integrated transcriptional and metabolic circuits**. *Front Bioeng Biotechnol* 2019, **7**:40.

59. Sarkar K, Chakraborty S, Bonnerjee D, Bagh S: **Distributed computing with engineered bacteria and its application in solving chemically generated 2 × 2 maze problems**. *ACS Synth Biol* 2021, **10**:2456-2464.

60. • Wang Y, An B, Xue B, Pu J, Zhang X, Huang Y, Yu Y, Cao Y, Zhong C: **Living materials fabricated via gradient mineralization of light-inducible biofilms**. *Nat Chem Biol* 2021, **17**:351-359.

In this paper, the authors developed patterned engineered living materials by coupling light-inducible bacterial biofilm formation with biomimetic hydroxyapatite (HA) mineralization. The location and the degree of mineralization were regulated by controlling the spatial and biomass density of the biofilm.

61. Duraj-Thatte AM, Manjula-Basavanna A, Courchesne N-MD, Cannici GI, Sánchez-Ferrer A, Frank BP, van't Hag L, Cotts SK, Fairbrother DH, Mezzenga R, *et al.*: **Water-processable, biodegradable and coatable aquaplastic from engineered biofilms**. *Nat Chem Biol* 2021, **17**:732-738.

62. Duraj-Thatte AM, Courchesne N-MD, Praveschotinunt P, Rutledge J, Lee Y, Karp JM, Joshi NS: **Genetically programmable self-regenerating bacterial hydrogels**. *Adv Mater* 2019, **31**:1901826.

63. Schaffner M, Rühs PA, Coulter F, Kilcher S, Studart AR: **3D printing of bacteria into functional complex materials**. *Sci Adv* 2017, **3**:eaao6804.

64. Gilbert C, Tang TC, Ott W, Dorr BA, Shaw WM, Sun GL, Lu TK, Ellis T: **Living materials with programmable functionalities grown from engineered microbial co-cultures**. *Nat Mater* 2021, **20**:691-700.

65. Caro-Astorga J, Walker KT, Herrera N, Lee K-Y, Ellis T: **Bacterial cellulose spheroids as building blocks for 3D and patterned living materials and for regeneration**. *Nat Commun* 2021, **12**:5027.

66. Chen B, Kang W, Sun J, Zhu R, Yu Y, Xia A, Yu M, Wang M, Han J, Chen Y, *et al.*: **Programmable living assembly of materials by bacterial adhesion**. *Nat Chem Biol* 2021, **18**:289-294.

67. Baldwin G, Bayer T, Dickinson R, Freemont PS, Ellis T, Kitney RI, Polizzi KM, Stan GB: **Synthetic Biology: A Primer - Modelling Synthetic Biology Systems**. Imperial College Press; 2016:89-107.

68. Sekine R, Shibata T, Ebisuya M: **Synthetic mammalian pattern formation driven by differential diffusivity of Nodal and Lefty**. *Nat Commun* 2018, **9**:5456.

69. Vittadello ST, Leyshon T, Schnoerr D, Stumpf MPH: **Turing pattern design principles and their robustness**. *Philos Trans R Soc A Math Phys Eng Sci* 2021, **379**:20200272.

70. Collier JR, Monk NA, Maini PK, Lewis JH: **Pattern formation by lateral inhibition with feedback: a mathematical model of delta-notch intercellular signalling**. *J Theor Biol* 1996, **183**:429-446.

71. Morsut L, Roybal KT, Xiong X, Gordley RM, Coyle SM, Thomson M, Lim WA: **Engineering customized cell sensing and response behaviors using synthetic notch receptors**. *Cell* 2016, **164**:780-791.

72. Toda S, Blauch LR, Tang SKY, Morsut L, Lim WA: **Programming self-organizing multicellular structures with synthetic cell-cell signaling**. *Science* 2018, **361**:156-162.

73. Matsuda M, Koga M, Woltjen K, Nishida E, Ebisuya M: **Synthetic lateral inhibition governs cell-type bifurcation with robust ratios**. *Nat Commun* 2015, **6**:6195.

74. Adams BL: **The next generation of synthetic biology chassis: moving synthetic biology from the laboratory to the field**. *ACS Synth Biol* 2016, **5**:1328-1330.

75. Gallup O, Ming H, Ellis T: **Ten future challenges for synthetic biology**. *Eng Biol* 2021, **5**:51-59.

76. Shen J, Liu F, Tu Y, Tang C: **Finding gene network topologies for given biological function with recurrent neural network**. *Nat Commun* 2021, **12**:3125.