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Towards synthetic microbial consortia for bioprocessing

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The use of microbial consortia for bioprocessing has been limited by our ability to reliably control community composition and function simultaneously. Recent advances in synthetic biology have enabled population-level coordination and control of ecosystem stability and dynamics. Further, new experimental and computational tools for screening and predicting community behavior have also been developed. The integration of synthetic biology with metabolic engineering at the community level is vital to our ability to apply system-level approaches to building and optimizing synthetic consortia for bioprocessing applications. This review details new methods, tools and opportunities that together have the potential to enable a new paradigm of bioprocessing using synthetic microbial consortia.

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Introduction

The biosynthesis of compounds of medical and industrial importance often requires engineering and optimization of complex metabolic pathways. Traditionally, these processes have employed a clonal population of recombinant microbes such as *Escherichia coli* or yeast. There are many limitations of using a single population that could be alleviated or addressed by using a mixed community of organisms, such as metabolic load and the number of exogenous elements that can be cloned and optimized in a single cell [1]. Another advantage of using microbial consortia is compartmentalization, where active or passive transport of substrates or intermediates across the cell membrane could be used to facilitate a decrease in undesired cross-reactions and side products. Finally, using microbial consortia can combine the catalytic specialties of different species to produce new products. This strategy is inspired by naturally occurring microbial consortia, where ubiquitous communities consist of multiple populations that coexist and carry out complex chemical processes and physiological functions to enable survival of the community [2,3].

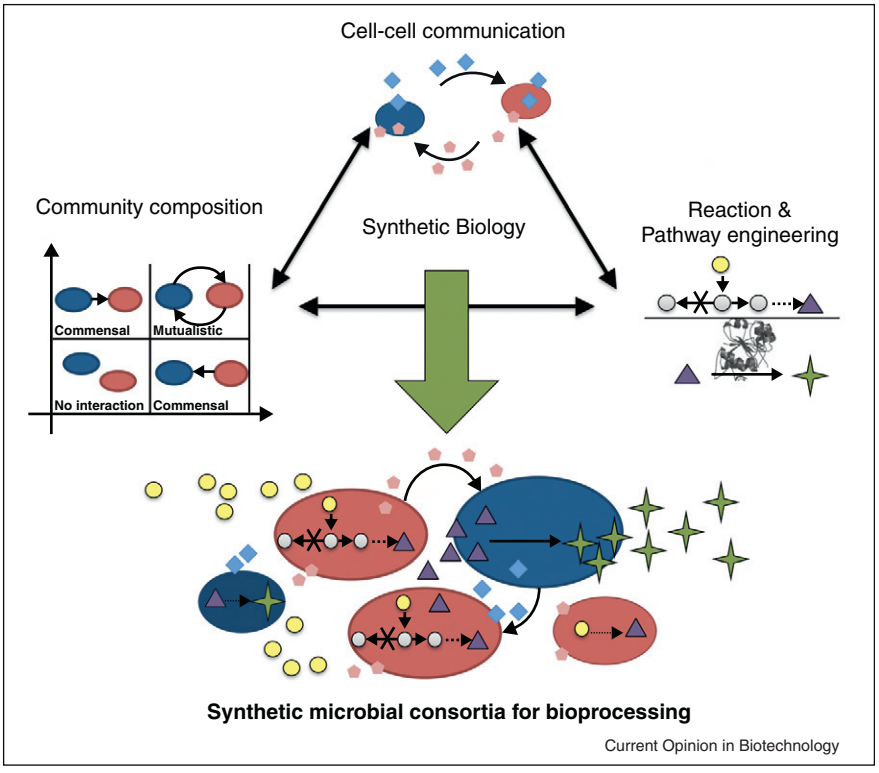
Early innovators in the fields of biochemical and biological engineering recognized the potential of microbial consortia for bioprocessing and biotechnological applications. Yet while there are rare examples of success (recently reviewed [4]), mono-culture systems continue to dominate the landscape of industrial bioprocessing. The major barrier to using communities for bioprocessing is that it requires simultaneous control of both the individual microbes and the ecosystem as a whole. For example, engineering individual microbes often leads to a change in their relative fitness and results in a change in community composition that can be detrimental to the overall process. Therefore, efforts to date have focused on engineering single microbes to efficiently carry out entire processes and on modifying the environment or culture conditions to improve yields from established microbial consortia.

We describe recent advances that will enable the future use of synthetic microbial communities for bioprocessing. This review focuses on the engineering of new biological components that enable cell–cell communication, the development of new strategies for enabling predictable ecosystem composition, and new biological tools that together represent essential elements for the successful implementation of a division of labor approach to bioprocessing using microbial consortia (see Figure 1).

Engineering communication

Ecosystem dynamics and stability are often modulated through interactions between organisms [5]. These interactions can be direct communication using signaling molecules or cell–cell contact, or they can be indirect, such as through the sharing of nutrients. One of the grand challenges in synthetic biology has been the ability to send signals between cells and to coordinate population-level behaviors. The most popular tools for engineering communication are based on quorum-sensing (QS) systems used by bacteria to sense and respond to changes in their local population density. Both the acyl-homoserine lactone (AHL)-based QS systems from Gram-negative organisms and the interspecies autoinducer-2 (AI-2) system have been engineered extensively [6]. Efforts include the directed evolution of the signal synthase [7], and signal sensitivity and specificity of the transcription factors that recognize the signals [8]. Roy and coworkers

Figure 1



Schematic of bioprocessing with synthetic microbial consortia. Engineering of cell–cell communication, community composition, and metabolic pathways are combined to enable coordination, division of labor, and product formation.

recently added the ability to turn off a QS response in the AI-2 system. Here the phosphorylation of AI-2 by extracellular LsrK quenches the QS response [9].

Synthetic cell–cell communication systems in yeast have also been described. An early example from Chen and Weiss used the production and recognition of a diffusible plant hormone from *Arabidopsis thaliana* to enable cell–cell communication and QS in *Saccharomyces cerevisiae* [10]. Groß *et al.* constructed a system where the roles of sensing and response were delegated to two populations in a coculture of *S. cerevisiae* [11]. In this case, they utilized a natural yeast pheromone, α-factor, to send or amplify a signal from one population to the next. This combination of modularity and cell–cell communication enables independent optimization of function in each strain. A second study used an α-factor-based system in the construction of a community capable of computing complex Boolean logic functions [12••]. First, a library of yeast cell modules that respond to an extracellular stimulus and/or α-factor and produce GFP as a reporter or α-factor to propagate the signal to the next population was constructed. The modules were successfully combined to produce 2-input and 3-input logic functions. This type of distributed computation could endow consortia with very useful and novel capabilities, such as enabling the system to adjust to different types of substrate and inhibitor mixtures.

Challenges in the area of cell–cell communication remain the limited number of independent communication modules, crosstalk between signals, and interspecies communication. The development of new signaling systems or modules is needed to address each of these challenges. The peptide-based QS systems used by Gram-positive organisms, where the high information content of the peptides could also limit crosstalk, remain untapped by synthetic biologists. Signals need not be limited to molecules that have been defined as QS inducers. Weber *et al.* engineered a system where volatile acetaldehyde was used to enable both intrakingdom and interkingdom communication between bacteria, yeast and mammalian cells [13]. While this work illustrates the potential to engineer communication across multiple cell types, new signaling systems should enable communication across species and kingdoms and include both diffusible and contact-based signals.

Engineering communities

An important practical constraint of employing microbial communities for bioprocessing is the ability to reliably generate stable or dynamic community behavior and

ecosystem composition. Early efforts by synthetic biologists showed that the control of toxic or savior proteins in combination with QS systems for signal propagation could enable a range of programmable ecosystems [14,15]. However, the success of these synthetic circuits is generally short-lived when cells are cultured outside of a microfluidic device, where larger cell populations increase the probability that a mutant capable of outcompeting the starting cells will arise [16]. While these studies have clearly established the potential for using synthetic biology to control community composition, new approaches are needed to enable coexistence at the scale required for bioprocessing applications.

One approach to enabling coexistence is to engineer beneficial interactions between each individual population. Several efforts have shown that mutualism can be achieved using combinations of auxotrophs. Shou *et al.* engineered two yeast strains that each coexist by supplying an essential metabolite to the other [17]. A mathematical model was built to analyze the requirements and constraints of the system. The initial growth rates and survival rate of both strains and their metabolite production rate were found to be critical for cooperative interactions to occur. A subsequent study used a series of 1035 *E. coli* auxotroph pairs to elucidate how different pairings can prove beneficial while others are not [18••]. Here, Wintermute and Silver showed that crossfeeding of metabolites yielded a significant metabolic synergy in 17% of pairings and constructed a quantitative model to describe and predict these synthetic interactions. Hu and coworkers recently combined the tuning of genetics, cell–cell communication and the environment to produce a range of population dynamics in a synthetic ecosystem, where two strains of *E. coli* directly modulate each other's growth via two AHL-based QS signal transduction circuits that control antibiotic resistance [19••]. They used a combination of computation and experiments to successfully identify combinations of AHL and antibiotics that produced specific dynamic ecosystem behaviors, including extinction, obligatory mutualism, facultative mutualism and commensalism.

Biofilms are of particular interest because these three-dimensional, surface-associated communities are often composed of multiple microbes and have potential for both bioremediation and bioprocessing applications [20]. Spatial heterogeneity, an important stabilizing force in microbial communities, has been investigated using synthetic bacterial communities [21–23]. An important hurdle is the ability to construct biofilms with defined community composition. Stubblefield and coworkers recently described a method for generating rationally assembled multispecies biofilms using the circulation of specific organism mixtures through a flow cell [24]. This sequential deposition approach has an advantage over other methods such as cell printing due to its simplicity and potential for scale-up. A synthetic QS-based communication circuit has also been used to program biofilm formation and dispersal [25].

Tools for enabling synthetic microbial consortia for bioprocessing

High-throughput screening methods for assessing community composition, dynamics and productivity are essential for the development of this field. Park and coworkers have demonstrated that microencapsulation of *E. coli* cocultures can be used to compartmentalize microbial populations in microdroplets and facilitate analysis of localized population-level behaviors [26••]. They constructed a synthetic consortia consisting of a tryptophan auxotroph and a tyrosine auxotroph. Significant growth as a result of crossfeeding was only observed in microdroplets containing both auxotrophs. Inkjet printing-based systems and other hydrogel encapsulation methods may be useful for building and characterizing synthetic communities [27,28].

A key variable to modifying ecosystem composition and stability is, of course, the environment. While engineering interactions between species that promote the desired community composition is an important tool for tuning community composition, altering the environment represents a complimentary approach. Brute force screening of different media can be used to determine conditions that promote coexistence and, ideally, product formation. Zhang *et al.* used this type of approach to identify a chemically defined medium for coculturing *Ketogulonicigenium vulgare* and *Bacillus megaterium* [29]. This pair of microbes is commonly used to produce 2-keto-L-gulonic acid (2-KLG), the immediate precursor of ascorbic acid (Vitamin C). While optimizing growth conditions through experimentally testing different conditions can lead to increased yields, the application of modern systems biology methods can provide new opportunities. For example, a combination of time series metabolic and proteomic profiling was recently used to elucidate interactions between *B. megaterium* and *K. vulgare* [30,31•]. They showed that intracellular metabolism and cell–cell communication via metabolic cooperation were essential in determining the population dynamics and productivity of the coculture.

Metabolic modeling and analysis methods must be adapted to capture the growth and productivity in microbial communities. Taffs *et al.* recently developed a compartmentalized model for analyzing cellular metabolic networks in microbial communities based on elementary mode analysis (EMA) in which each clonal population was treated as a distinct compartment and exchangeable metabolites were transferred through a fourth compartment representing the extracellular environment [32]. While this approach described and explained the mass and energy flows observed in a natural consortia, it is

computationally expensive and requires a great deal of *a priori* knowledge. Interestingly, a nested approach, where successive rounds of EMA identify potential interactions within a consortium, produced similar results with respect to the limits of the solution space. Klitgord and Segre conducted flux balance analysis (FBA) to predict microbial interactions and growth in different environmental conditions [33••]. They employed a constraint-based mathematical model to span a broad range of growth conditions and predict interactions between different microbes. They were able to verify their results from a previous literature studying existing complex interactions, demonstrating that it is possible to identify optimal growth conditions that induce mutualistic or commensal interactions between any two species.

Microbial consortia for bioprocessing

The field of biofuel production is ripe with opportunities to use cocultures, where a bioprocessing approach that converts biomass to biofuel in a single reactor has significant potential for producing low-cost biofuel [34]. Two strains of *E. coli* were recently constructed that cooperate in the transformation of xylan into ethanol [35], where one strain secretes two hemicellulases while the other uses the released sugars to produce ethanol. The control single culture containing the expression of both parts proved to have a lower yield of ethanol compared to the binary culture. The challenges observed in this work with regard to balancing the populations of the two strains illustrates the need for considering both function and ecology. Chen and coworkers' recent work using synthetic yeast consortia to produce ethanol from cellulose demonstrates the potential of a division of labor approach [36,37••]. Here, three different yeast strains were developed to secrete three different proteins with docking-tags enabling their assembly onto an extracellular scaffold. The three specific heterologous enzymes were an endoglucanase (AT), an exoglucanase (CB) and a β-glucosidase (BF) and together are capable of cellulose degradation. The consortia population was modulated by adjusting the inoculation ratio of each of the four strains including the Scaf-ctf producing strain (SC). The final reported ratio was 7:2:4:2 of SC:AT:CB:BF. This optimized ratio produced 87% of the theoretical ethanol production value from phosphoric acid swollen cellulose (PASC), and was 3-fold higher than a similar consortium producing the secreted enzymes only with a control strain (CE) in place of SC. Most importantly, the differences in cell growth cannot explain the 3-fold increased ethanol production. Opportunities to improve on established cocultures are not limited to biofuel production. Examples describing the use of microbial consortia for bioremediation [38], lipid production [39], and biopolymer production [40] have been recently reported (reviewed in [4]), and represent new opportunities to apply synthetic biology tools to build synthetic microbial consortia for a variety of bioprocessing applications.

Conclusions and perspectives

New tools for the analysis and engineering of microbial communities have been developed that together represent a framework for engineers to begin to apply synthetic biology and metabolic engineering approaches to microbial consortia. However, there are many challenges that must be addressed before microbial communities are likely to become commonplace in industrial bioprocessing. Reliable community behavior remains an important challenge in this area. Recent studies indicate that microbes may be primed to coexist [41] and novel computational and experimental systems exploring biodiversity [42,43] may provide a new set of tools for constructing synthetic consortia. The new methods described above that use metabolic network information to predict media conditions that promote coexistence of strains represent an important advance. Further improvements to our ability to model and optimize metabolic pathways for targeted product formation across a community are essential and we anticipate that recent interest in this area will lead to new developments in the near future.

The biosynthetic potential of synthetic microbial consortia represents both exciting opportunities and challenges that require system-level approaches. As such, this emerging area holds great promise for not only bioprocessing, but also bioremediation, biosensing and other applications where microbial consortia can enable complex behaviors through the combined strengths of the individual organisms.

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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. Brenner K, You L, Arnold FH: **Engineering microbial consortia: a new frontier in synthetic biology**. *Trends Biotechnol* 2008, **26**:483-489.

2. Keller L, Surette MG: **Communication in bacteria: an ecological and evolutionary perspective**. *Nat Rev Microbiol* 2006, **4**:249-258.

3. Pai A, Tanouchi Y, Collins CH, You L: **Engineering multicellular systems by cell–cell communication**. *Curr Opin Biotechnol* 2009, **20**:461-470.

4. Sabra W, Dietz D, Tjahjasari D, Zeng A-P: **Biosystems analysis and engineering of microbial consortia for industrial biotechnology**. *Eng Life Sci* 2010, **10**:407-421.

5. Wintermute EH, Silver PA: **Dynamics in the mixed microbial concourse**. *Genes Dev* 2010, **24**:2603-2614.

6. Hooshangi S, Bentley WE: **From unicellular properties to multicellular behavior: bacteria quorum sensing circuitry and applications**. *Curr Opin Biotechnol* 2008, **19**:550-555.

7. Kambam PKR, Sayut DJ, Niu Y, Eriksen DT, Sun L: **Directed evolution of LuxI for enhanced OHHL production**. *Biotechnol Bioeng* 2008, **101**:263-272.

8. Collins CH, Leadbetter JR, Arnold FH: **Dual selection enhances the signaling specificity of a variant of the quorum-sensing transcriptional activator LuxR**. *Nat Biotechnol* 2006, **24**:708-712.

9. Roy V, Fernandes R, Tsao C-y, Bentley WE: **Cross species quorum quenching using a native AI-2 processing enzyme**. *ACS Chem Biol* 2010, **5**:223-232.

10. Chen MT, Weiss R: **Artificial cell–cell communication in yeast *Saccharomyces cerevisiae* using signaling elements from *Arabidopsis thaliana***. *Nat Biotechnol* 2005, **23**:1551-1555.

11. Groß A, Rödel G, Ostermann K: **Application of the yeast pheromone system for controlled cell–cell communication and signal amplification**. *Lett Appl Microbiol* 2011, **52**:521-526.

12. •• Regot S, Macia J, Conde N, Furukawa K, Kjellen J, Peeters T, Hohmann S, de Nadal E, Posas F, Solé R: **Distributed biological computation with multicellular engineered networks**. *Nature* 2011, **469**:207-211.

Yeast consortia were engineered to perform complex Boolean logic functions by compartmentalizing simple logic functions in individual strains and connecting them via cell–cell communication.

13. Weber W, Daoud-El Baba M, Fussenegger M: **Synthetic ecosystems based on airborne inter- and intrakingdom communication**. *Proc Natl Acad Sci USA* 2007, **104**:10435-10440.

14. Balagadde FK, Song H, Ozaki J, Collins CH, Barnet M, Arnold FH, Quake SR, You LC: **A synthetic *Escherichia coli* predator–prey ecosystem**. *Mol Syst Biol* 2008, **4**:187.

15. You LC, Cox RS, Weiss R, Arnold FH: **Programmed population control by cell–cell communication and regulated killing**. *Nature* 2004, **428**:868-871.

16. Balagaddé FK, You L, Hansen CL, Arnold FH, Quake SR: **Long-term monitoring of bacteria undergoing programmed population control in a microchemostat**. *Science* 2005, **309**:137-140.

17. Shou W, Ram S, Vilar JMG: **Synthetic cooperation in engineered yeast populations**. *Proc Natl Acad Sci USA* 2007, **104**:1877-1882.

18. •• Wintermute EH, Silver PA: **Emergent cooperation in microbial metabolism**. *Mol Syst Biol* 2010, **6**:407.

Auxotrophic microorganisms were screened to elucidate how different pairs interact, and 17% of interactions were observed to be synergistic. A stoichiometric model that captures and predicts synergistic interactions was described.

19. •• Hu B, Du J, Zou R-y, Yuan Y-j: **An environment-sensitive synthetic microbial ecosystem**. *PLoS One* 2010, **5**:e10619.

This work describes a synthetic microbial community where environmental conditions can be tuned to promote a range of ecosystem behaviors.

20. Wood TK, Hong SH, Ma Q: **Engineering biofilm formation and dispersal**. *Trends Biotechnol* 2011, **29**:87-94.

21. Kim HJ, Boedicker JQ, Choi JW, Ismagilov RF: **Defined spatial structure stabilizes a synthetic multispecies bacterial community**. *Proc Natl Acad Sci USA* 2008, **105**:18188-18193.

22. Song H, Payne S, Gray M, You L: **Spatiotemporal modulation of biodiversity in a synthetic chemical-mediated ecosystem**. *Nat Chem Biol* 2009, **5**:929-935.

23. Brenner K, Arnold FH: **Self-organization, layered structure, and aggregation enhance persistence of a ynthetic biofilm consortium**. *PLoS One* 2011, **6**:e16791.

24. Stubblefield Ba, Howery KE, Islam BN, Santiago AJ, Cardenas WE, Gilbert ES: **Constructing multispecies biofilms with defined compositions by sequential deposition of bacteria**. *Appl Microbiol Biotechnol* 2010, **86**:1941-1946.

25. Hong SH, Hegde M, Kim J, Wang X, Jayaraman A, Wood TK: **Synthetic quorum-sensing circuit to control consortial biofilm formation and dispersal in a microfluidic device**. *Nat Commun* 2012, **3**:613.

26. •• Park J, Kerner A, Burns Ma, Lin XN: **Microdroplet-enabled highly parallel co-cultivation of microbial communities**. *PLoS One* 2011, **6**:e17019.

The authors developed a coculture system for screening microbial communities where microdroplets compartmentalize communities and enable high-throughput screening.

27. Choi WS, Ha D, Park S, Kim T: **Synthetic multicellular cell-to-cell communication in inkjet printed bacterial cell systems**. *Biomaterials* 2011, **32**:2500-2507.

28. Choi WS, Kim M, Park S, Lee SK, Kim T: **Patterning and transferring hydrogel-encapsulated bacterial cells for quantitative analysis of synthetically engineered genetic circuits**. *Biomaterials* 2012, **33**:624-633.

29. Zhang J, Zhou J, Liu J, Chen K, Liu L, Chen J: **Development of chemically defined media supporting high cell density growth of *Ketogulonicigenium vulgare* and *Bacillus megaterium***. *Bioresour Technol* 2011, **102**:4807-4814.

30. Zhou J, Ma Q, Yi H, Wang L, Song H, Yuan Y-J: **Metabolome profiling reveals metabolic cooperation between *Bacillus megaterium* and *Ketogulonicigenium vulgare* during induced swarm motility**. *Appl Environ Microbiol* 2011, **77**:7023-7030.

31. • Ma Q, Zhou J, Zhang W, Meng X, Sun J, Yuan Y-j: **Integrated proteomic and metabolomic analysis of an artificial microbial community for two-step production of vitamin C**. *PLoS One* 2011, **6**:e26108.

Combined proteomic and metabolic profiles of an artificial microbial community were used to elucidate intercellular interactions to optimize growth conditions.

32. Taffs R, Aston JE, Brileya K, Jay Z, Klatt CG, McGlynn S, Mallette N, Montross S, Gerlach R, Inskeep WP *et al.*: **In silico approaches to study mass and energy flows in microbial consortia: a syntrophic case study**. *BMC Syst Biol* 2009, **3**:114.

33. •• Klitgord N, Segre D: **Environments that induce synthetic microbial ecosystems**. *PLoS Comp Biol* 2010, **6**:e1001002.

The authors developed algorithms to predict optimal environmental conditions for promoting coexistence in synthetic microbial communities.

34. Lynd LR, Laser MS, Bransby D, Dale BE, Davison B, Hamilton R, Himmel M, Keller M, McMillan JD, Sheehan J, Wyman CE: **How biotech can transform biofuels**. *Nat Biotechnol* 2008, **26**:169-172.

35. Shin H-D, McClendon S, Vo T, Chen RR: ***Escherichia coli* binary culture engineered for direct fermentation of hemicellulose to a biofuel**. *Appl Environ Microbiol* 2010, **76**:8150-8159.

36. Goyal G, Tsai S-L, Madan B, DaSilva N, Chen W: **Simultaneous cell growth and ethanol production from cellulose by an engineered yeast consortium displaying a functional mini-cellulosome**. *Microb Cell Fact* 2011, **10**:89.

37. •• Tsai S-L, Goyal G, Chen W: **Surface display of a functional minicellulosome by intracellular complementation using a synthetic yeast consortium and its application to cellulose hydrolysis and ethanol production**. *Appl Environ Microbiol* 2010, **76**:7514-7520.

The authors developed a synthetic yeast consortium that produced a higher yield of ethanol with an optimal ratio of cells.

38. Li L, Yang C, Lan W, Xie S, Qiao C, Liu J: **Removal of methyl parathion from artificial off-gas using a bioreactor containing a constructed microbial consortium**. *Environ Sci Technol* 2008, **42**:2136-2141.

39. Xue F, Miao J, Zhang X, Tan T: **A new strategy for lipid production by mix cultivation of *Spirulina platensis* and *Rhodotorula glutinis***. *Appl Biochem Biotechnol* 2010, **160**:498-503.

40. Moralejo-Gárate H, Mar'atusalihat E, Kleerebezem R, van Loosdrecht M: **Microbial community engineering for biopolymer production from glycerol**. *Appl Microbiol Biotechnol* 2011, **92**:631-639.

41. Hosoda K, Suzuki S, Yamauchi Y, Shiroguchi Y, Kashiwagi A, Ono N, Mori K, Yomo T: **Cooperative adaptation to establishment of a synthetic bacterial mutualism**. *PLoS One* 2011, **6**:e17105.

42. Ramadas R, Thattai M: **Flipping DNA to generate and regulate microbial consortia**. *Genetics* 2010, **184**:285-293.

43. Sekine R, Yamamura M, Ayukawa S, Ishimatsu K, Akama S, Takinoue M, Hagiya M, Kiga D: **Tunable synthetic phenotypic diversification on Waddington's landscape through autonomous signaling**. *Proc Natl Acad Sci USA* 2011, **108**:1-5.