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Engineering multicellular systems by cell-cell communication

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Abstract

Synthetic biology encompasses the design of new biological parts and systems as well as the modulation of existing biological networks to generate novel functions. In recent years, increasing emphasis has been placed on the engineering of population-level behaviors using cell-cell communication. From the engineering perspective, cell-cell communication serves as a versatile regulatory module that enables coordination among cells in and between populations and facilitates the generation of reliable dynamics. In addition to exploring biological “design principles” via the construction of increasingly complex dynamics, communication-based synthetic systems can be used as well-defined model systems to study ecological and social interactions such as competition, cooperation and predation. Here we discuss the dynamic properties of cell-cell communication modules, how they can be engineered for synthetic circuit design, and applications of these systems.

1. Introduction

Synthetic biology aims to generate well-defined controllable functions in living organisms. The design of such functions involves using well-characterized biological parts from natural systems, manipulating existing cellular networks, or creation of new parts altogether [1,2], to form new genetic networks called “synthetic circuits”. This past decade has seen an explosion in the design of such synthetic circuits with increasing complexity and a rapid expansion of engineered functions [3–5]. The ability to generate desired functionality using synthetic circuits is useful in a wide variety of applications from therapeutics to green chemistry [5,6]. In addition, the use of existing biological parts in synthetic circuits provides vital insights into the roles of these parts in their natural context.

Cell-cell communication leading to multicellular behavior has attracted great attention for use in synthetic circuits. In nature, communication is critical for the physiological functions of diverse organisms. In the nervous system, activity-dependent ATP release by nervous system cells acts as an extracellular signal detected by purinergic membrane receptors that modulate intracellular calcium and cyclic AMP [7]. In this case, cell-cell communication links together a wide variety of cells essential to the functioning of the nervous system within a complex organism. During development in *Drosophila*, intercellular signaling combines with spatial signal gradient sensing for the formation of vein structure on the wing [8]. Here, a well-defined,

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multicellular pattern emerges from the controlled action of individual cells. In bacteria, populations monitor their own density and achieve coordinated expression of target genes in a phenomenon known as quorum sensing (QS) [9]. The coordination that results from QS-dependent communication is postulated to benefit the population as a whole [10]. In each case, cell-cell communication is essential to the formation of a well controlled, coordinated, multicellular system. These properties make communication an important feature for synthetic circuits.

Natural communication modules, especially those from bacteria [11], have been exploited to program synthetic population behavior [3,12]. Here, communication is typically via diffusible chemicals that are synthesized and detected by individual cells where they alter downstream gene expression. Other cellular modules, such as metabolic networks, have also been re-engineered to realize communication [13]. To encompass all these efforts, we define 'communication' simply as any interaction between cells where the sender, the carrier and the receiver of specific information can be identified [14,15]. Here we discuss the basic properties of cell-cell communication, the design of communication-based multicellular systems and their applications.

2. Cell-cell communication and its properties

The natural QS systems in Gram-negative bacteria often use acyl homoserine lactones (AHLs) as communication signals [16,17]. These AHLs are typically synthesized by LuxI-type enzymes from fatty acids, where LuxI is the canonical AHL synthase from Gram-negative bacterium *Vibrio fischeri*. Gram-positive bacteria often use small peptides as the QS signals [18,19]. In all QS systems, signals are produced intracellularly and transported to the extracellular environment. The smaller AHLs diffuse freely across bacterial cell membranes [20] while peptides and large AHLs appear to be actively transported by pumps [19,21,22]. These signals are detected by different strategies; AHL signals often lead to activation of cytoplasmic regulator proteins such as LuxR in *V. fischeri* [17,20], which then activates target gene expression. Peptide signals and also some AHLs, are typically sensed by membrane-associated receptors to initiate a phosphorylation cascade that leads to target gene expression [18,19]. The list of target genes under QS control is diverse, such as bioluminescence in *Vibrio harveyi* [23], competence regulation in *Streptococcus pneumoniae* [24], exoenzyme secretion in *P. aeruginosa* and other plant pathogens [25,26], conjugation in *Agrobacterium tumefaciens* [27] and virulence in *Staphylococcus aureus* [28].

To date, most communication-based synthetic circuits have exploited bacterial QS especially those from Gram-negative bacteria. These systems are tremendously diverse in terms of their sensory components, the biochemical and transport properties of signaling molecules, and the genes and functions that are controlled in a density-dependent manner. We illustrate their general functioning with a minimal motif comprised of signal synthesis, secretion, degradation and detection elements (Fig. 1a), and use the *lux* system of *V. fischeri* as a canonical example. At low cell density, the AHL concentration is low both inside and outside of the cells. As cell density increases, the local AHL concentration increases. Within the cells, the cytoplasmic transcription factor LuxR recognizes AHL and activates gene expression of the well-characterized *luxI* promoter (P_{luxI}) [29]. Therefore, the expression of P_{luxI} is correlated to local population density through the production and detection of the AHL signal molecule.

We define a d_{crit} as the critical cell density at which a threshold concentration (K) of signal is reached (Fig. 1b), where K is commonly defined as the signal concentration at which target gene expression is half maximal (Fig. 1b inset). This d_{crit} acts as a simple measure of the module's characteristics and can be derived in terms of the basic QS parameters: signal synthesis, secretion, degradation and detection [30]. Increasing signal synthesis while

decreasing signal diffusion, degradation and threshold, decrease d_{crit} . That is, doing any of this will lower the density at which the population activates (Fig. 1b). We can also use an alternative perspective of a single cell and define a metric ‘sensing potential’ (v) that, instead of a critical density, considers the microenvironment size V_e that results in the activation of an individual

confined cell [30]. Mathematically, $v = \frac{V_e}{V_c}$ where V_c is the cell volume and V_e the critical microenvironment size for activation. v is equivalent to d_{crit} as density can be interpreted in terms of average volume available to each cell giving $v = 1/(d_{crit}V_c)$. However, v allows a consistent connection between an individual QS cell’s activation and its population-level phenotype and is, in that sense, more general.

The metric and equations show that if signal synthesis is too high compared to its rate of diffusion, then signal accumulation within the cells can result in the target gene expression being ON (above half-maximal) *regardless of cell density* [30]. When this ‘self-activation’ occurs, the role of communication-based activation is reduced (in terms of increase in fold target induction from low to high cell density).

Other variations in QS modules, such as when the *extracellular* signal is sensed by cell surface receptors [9,19], when there is positive feedback on signal synthesis [31], or when signal molecules are actively transported by pumps rather than by passive diffusion [22], can similarly be accounted for and in each case, the relationships between d_{crit} and the system parameters can be derived [30].

A salient characteristic of many QS modules is the stabilization of ‘LuxR’ homologs (R-protein) upon AHL binding [32,33]. As an example, the R-protein TraR from *A. tumefaciens* is extremely unstable with a half-life of 2–3 min and binding its cognate signal increases its half-life by 25-fold [32]. This instability appears to be wasteful as cells would have to synthesize it with a higher rate to sustain R-protein levels. Recent theoretical studies indicate at least two potential roles for R-protein instability. First, an unstable R-protein could reduce variability in QS circuit behavior [34] (Fig. 1c). Gene expression is intrinsically noisy, primarily due to small numbers of interacting molecules within cells and fluctuations in environmental conditions [35]. The fast turnover of R-proteins together with AHL diffusion across cell membrane is predicted to reduce variability in QS-mediated target gene expression, via a mechanism termed “diffusional dissipation” [34].

A second potential role for R-protein instability is signal discrimination [36]. In nature, diverse AHL-dependent QS systems exist within and among different species of bacteria. While R-proteins generally have a strong preference for their native AHL signal, at high concentrations they can bind non-cognate AHLs and induce gene expression, a phenomenon typically termed “crosstalk”. This implies that bacteria would need some mechanism to differentiate between their cognate and other non-cognate AHL signals to minimize crosstalk. One way to do this is to have asymmetry between the binding reactions of the two to the R-protein. An asymmetry could also exist in the subsequent dimerization of their R-protein-AHL complex [37,38]. This sequence of reactions constitutes a mechanism analogous to the canonical Hopfield-Ninio model of kinetic proofreading [39,40] (Fig. 1d). Smith *et al.* [36] proposed that asymmetry in another step, stabilization of the R-protein by signal binding, adds an additional layer to the kinetic proofreading. That is, given the asymmetry in binding reactions, signal crosstalk can be further reduced when the cognate signal stabilizes R-protein to a higher degree than the non-cognate signal. Importantly, the effect of this asymmetry in R-protein stabilization is amplified if the R-protein is unstable. Although these features of R-protein dynamics remain to be tested experimentally, these theoretical studies [34,36] have shed light on possible consequences of R-protein instability.

By themselves, the coordination, noise reduction and signal discrimination properties of communication could be useful in synthetic circuits. Koseska *et al.* [41] modeled theoretical populations where cellular decision making is governed by multi-stable genetic switches. These switches are particularly susceptible to noise induced fluctuations, causing random jumps between alternative cellular states. The study shows that cell-cell communication in a population allows reliable synchronization of decision making among individuals, even in the presence of noise. Similarly, the synthetic gene circuit from Elowitz and Leibler [42] (the ‘repressilator’) effectively generates oscillations but these are highly variable and show rapid loss of synchrony between cells. Computational studies indicate that cell-cell communication could synchronize the oscillations and make them more robust to perturbations [43,44]. This communication induced oscillation synchrony remains to be demonstrated experimentally. However, robust oscillations using cell-cell communication circuits have been demonstrated in a microchemostat [45] using a version of the population controller (described below) [46].

3. Engineering the communication toolkit

Several well characterized natural communication modules [12] are now widely used in synthetic circuit design. However, a large pool of natural communication modules still remain to be explored, such as the oligopeptide-based QS systems in Gram-positive bacteria [19]. The autoinducer-2 (AI-2) QS signal [47,48] can act as an intra-species [49,50] or inter-species signal [51] and has only recently been incorporated into synthetic circuits [52].

Engineering these circuits involves the ad hoc choice of different QS elements and their optimization, depending on considerations such as “cross-talk”, and critical activation density (d_{crit}). As an example, consider the design of a microbial consensus consortium (MCC) by Brenner *et al.* [53] (Fig. 3c). Here, the *lasRI* and *rhIRI* systems from *P. aeruginosa* were used for bidirectional communication between two populations. These two systems did show low-level crosstalk but the authors used detailed circuit modeling to arrive at an architecture that maximized the consensus population response while minimizing any response due to crosstalk. Another strategy is to specifically engineer the R-protein for desired properties. Collins *et al.* [33] used a dual selection strategy to engineer LuxR mutants that respond to different AHLs with varying specificity. LuxR variants with increased affinity for a broad range of AHLs were also generated [54]. Such strategies provide a method to engineer parts that possess or lack a specific response to a signal. One of the engineered LuxR mutants was instrumental in the development of a ‘band-pass’ biological circuit [55]. Modulating the R-protein degradation rate without compromising binding characteristics could also improve signal discrimination [36].

Analysis of the minimal QS motif can guide the modulation of d_{crit} , which can be decreased by increasing signal production, decreasing signal degradation, transport or the threshold for activation (Fig. 1b). Signal production can be modulated by controlling the induction of its synthase gene. Signal degradation can be modulated by controlling the pH of the culture medium [46,56] while the threshold can be changed by using different R-protein variants [33,54,57] or by changing the signal inducible promoter (P_{luxI}) region. The dynamic properties of the individual elements themselves, such as the signal synthesis rate from a synthase, can also be systematically engineered. Kambam *et al.* [58,59] have used directed evolution to generate LuxI and RhlI mutants that display increased signal production rate compared to wild-type AHL synthases.

Existing cellular modules can also be co-opted to realize communication. Bulter *et al.* [13] reengineered carbon metabolism in *Escherichia coli* such that acetate, a commonly secreted metabolite, acts as a density dependent signal and parts of the nitrogen starvation regulon act as detectors to achieve cell-cell communication. Chen and Weiss [60] created a QS circuit in

yeast by using a signal (IP, a cytokinin) and receptor (AtCRE1) from *Arabidopsis thaliana* (Fig. 2d), demonstrating that communication circuits can also be generated by mixing and matching components from different natural systems.

4. Communication systems and their applications

Natural QS systems are typically much more complex than illustrated in Fig. 1a and much effort has been put into dissecting their dynamics [61]. These studies reveal the substantial complexity in commonly used QS modules. An alternative, therefore, is to examine QS dynamics in well-defined synthetic gene circuits with QS components. Haseltine and Arnold [62] examined the *luxRI* system regulation characteristics by rearranging the individual components of the system. They observed that target function activation could be either graded (linearly increasing with cell density), threshold (all or none above or below a critical cell density) or bistable (have a different threshold depending on initial conditions), depending on the arrangement.

In single populations, by using different target functions under QS control, interesting circuits have been illustrated [63,64]. A population controller [46] uses the *luxRI* system to achieve autonomous control of *E. coli* density. Here, a toxic protein, CcdB, is placed under the control of P_{luxI} , conferring a density-dependent increase in CcdB expression (Fig. 2a). The result is an increase in growth inhibition as cell density increases, leading to a controllable steady state cell density. Analogous to d_{crit} (Fig. 1c), this steady state can be modulated by changing the communication module parameters such as signal degradation rate [46]. The invasin circuit [65] is based on the observation that many bacterial species, including *E. coli*, have been observed to localize to tumors at high densities following intravenous injection [66]. Anderson *et al.* [65] surmised that this localization could act as a feature to identify tumors and invade them with engineered *E. coli*. Their design uses the *luxRI* system to control the invasin gene that enables *E. coli* to invade mammalian cells (Fig. 2b). *In vitro* studies showed that invasion of cancer-derived cells by *E. coli* carrying the circuit took place only when the inoculated *E. coli* were at high density. While the study does not directly contrast the cell-density based tumor invasion with other localization cues such as hypoxia, it demonstrates that autonomous communication based regulation could be useful in therapeutic applications.

A single cell-cell communication system can be used to design multiple communicating species by placing the signal synthesis and response elements into different cells, generating a sender and receiver respectively. This strategy was used in the design of a biological ‘pulse’ generator [67] and a ‘band-pass’ filter [55] in *E. coli*. In the latter study, sender cells express *luxI* and synthesize the diffusible AHL signal while receiver cells contain a circuit with the response elements *luxR*, promoter P_{luxI} , and repressors CI, LacI and LacI_{M1} (a LacI mutant) (Fig. 3a). The receiver circuit logic allows for GFP expression only when the AHL concentration is within a narrow range. On a solid surface, the diffusing signals form a spatial concentration gradient around sender cells. The distance of receivers from senders determines the signal concentration they encounter and hence also their response. The result is fascinating spatial patterns depending on the arrangement of senders and receivers. The authors combined experimental observations with simulations of pattern formation to study the roles of circuit parameters. The decay rate of LacI emerged as the major determinant of the time to pattern formation as well as any shift of the pattern itself. Signal production and sensing can be combined with other synthetic genetic parts in new and interesting ways as demonstrated recently by the construction of a biological ‘edge detector’ circuit [68]. Here, signal production in the circuit was tied to a light sensor and a genetic ‘inverter’ circuit to generate a variant of an AND gate. This logic programmed the cells to produce a pigment (through LacZ expression) only at the edge of light and dark regions. Similar studies on artificial pattern formation systems could aid investigations [69,70] into natural pattern formation questions, such as the surprising

robustness [71] in morphogen gradient-based patterning observed in *Drosophila* embryos despite large variations in the gradient profiles in individual embryos. Similarly, the patterns formed by swarming microbes [72,73] could be investigated by using circuits that tie the motility of cells with communication [74,75]. Overall, we see that signal production and its detection - as separately controlled parts-combined with the diffusion properties of the signal and other genetic parts can yield numerous applications.

The strategy of separating signal synthesis and signal detection was also used by Chuang *et al.* [76] to create a heterogeneous population of cooperating and non-cooperating cells (Fig. 3b). In cooperators, the *rhlRI* system controls the induction of an antibiotic resistance gene, the corresponding antibiotic of which is present in the media. Non-cooperators carry the same circuit except for the *rhlI* gene. In the combined population, the AHL produced by the cooperators acts as public goods for non-cooperators to survive as well. The authors considered a scenario where the overall population consists of subpopulations of different sizes with varying ratios of cooperators to non-cooperators. Within any subpopulation, non-cooperators benefit from public goods without paying any cost of its synthesis and thus grow faster than cooperators. Despite this, in some cases, the overall proportion of producers in the overall population can actually increase with time; a paradoxical statistical effect known as Simpson's paradox. A large variance between the initial subpopulation compositions is required for this effect to happen. The study shows that such large variance can be simply generated by large dilutions of the subpopulations resulting in stochastic fluctuations in compositions among them. Overall, the study provides a simple scenario where a trait beneficial to the population can emerge despite it not being favorable at the individual level. This design also offers the ability to tune various aspects of cooperation, such as changing cost and benefit by replacing the antibiotic resistance with another costly but beneficial system, and could be used to study other aspects such as the conditions for cooperation and its stability [77]. In self-destructive cooperation, cooperators die to benefit the remaining members of their population [78]. To address this, the same circuit could be modified to force the cooperators to commit suicide [46].

Multiple communicating populations can also be created by using two or more communication systems. In the two-population MCC system [53] (Fig. 3c), each population expresses its reporter only when the other is present at sufficiently high density, resulting in a population-level AND gate. The system presents the possibility of dividing a complex task among different microbial populations (division of labor) which coordinate in its execution [79]. Balagadde *et al.* [80] used the *luxRI* and *lasRI* systems in combination with a toxin and an anti-toxin to generate predator and prey populations (Fig. 3d). Depending on the operating conditions, the predator-prey populations display diverse ecological consequences such as species extinction, coexistence, as well as oscillations that can be predicted by mathematical modeling. By modulating growth rate, death rate and the strength of cell-cell communication, their effects on the interaction consequences can be studied. The same circuits have also been used to explore the maintenance of biodiversity in chemically-mediated ecosystems (H. Song *et al.*, unpublished). Synthetic communication circuits can thus be convenient tools to study biological questions [81] such as various aspects of cooperation (as seen in the previous paragraph) as well as species interactions.

Lastly, two or more populations can also be linked via an essential chemical (instead of an explicit signal). Weber *et al.* [82] used this approach to engineer inter- and intra-kingdom interactions with a variety of behaviors: predator-prey, commensalism, amensalism, parasitism and cooperation. In the predator-prey case, wild-type *E. coli* (predator) and Chinese hamster ovary (CHO) cells (prey) that constitutively express the ampicillin-degrading enzyme β -lactamase were used (Fig. 3e). Growth of the CHO cells is inhibited by rapid growth of *E. coli* in the same environment. In turn, *E. coli* cells, which are sensitive to ampicillin, require

sufficient number of CHO cells to sustain their growth in a medium with ampicillin. Shou *et al.* [83] similarly co-opted an essential chemical requirement to engineer cooperation between two yeast populations (Fig. 3f). Here, one strain requires the external supply of adenine and the other lysine. The adenine-requiring strain overproduces lysine and the lysine-requiring strain overproduces adenine so each can supply the essential metabolite for the other. The study points out that factors such as starvation tolerance, nutrient release timing and the initial population ratios of the two strains can restrict cooperation. Importantly, it also observes that the mutualistic and obligatory population is stable to sudden population reductions. The approach of linking individual cells via a diffusible chemical was also recently used to generate tunable band-pass circuits causing spatial patterns [84,85]. Here, the ‘band’ was defined by a zone of appropriate antibiotic concentration in which cells could grow while the position and width of the band could be externally tuned using ampicillin and tetracycline. Thus, just as was seen with communication based circuits earlier, these circuits that are linked via an essential chemical rather than an explicit signal, can similarly be used to study various biological questions.

Conclusions and Prospects

An impressive number of multicellular systems have been built in the past few years using natural or synthetic communication modules. However, a large number of natural communication systems, particularly non-bacterial ones, remain to be explored and adopted into the synthetic biology framework. We have listed several of their applications, stretching from therapeutics to replicating social behavior. Many other uses will emerge from tying communication to the power of synthetic biology and metabolic engineering. Autonomous density sensing can be coupled with motility control to realize bio-computation in the spatial domain [86]. Tasks that involve many reaction steps such as breaking down a complex substance in bioremediation [87] could be divided into several interacting populations that perform it under a division of labor [5,15]. Different microbes possess their own unique abilities and it may be easier to make them communicate and coordinate on a task rather than force the required gene sets from each into a single organism. The autonomous population control possible by communication (Fig. 3) can be combined with metabolic engineering to optimize the growth of circuit carrying microbes in bioreactors [5,88]. Engineering multicellular behavior thus displays a compelling range of applications.

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(a)

(b)

(c)

(d)

Fig. 1. Cell-cell communication and its properties

Single black arrows indicate reactions, double headed arrows indicate diffusion, grey arrows indicate activation and grey blunt arrows indicate inhibition.

(a) A minimal QS module.

(b) Target expression versus cell density. Each curve represents the action of cell-cell communication as in (a) but with different parameters. K refers to the threshold signal concentration leading to 50% target expression (inset). The effect of parameters is indicated. Vertical stippled lines for each case marks the critical density at which signal concentration exceeds K . Note that increasing k , decreasing D and d_a lower the critical density (lower d_{crit}) for target expression. Lowering K (horizontal stippled line) decreases d_{crit} too.

(c) Noise reduction by quorum sensing. Histogram of activated LuxR for unstable (blue) and stable (red) LuxR. Y axis shows the frequency with which corresponding number of activated LuxR molecules on the x-axis is observed over time. Each histogram is generated from a time course simulation of the minimal QS system (inset). Parameters are chosen so that the mean number of activated LuxR is the same in either case. Unstable LuxR reduces noise, resulting

in a tighter control on the number of activated LuxR molecules than in the case without diffusion. See Tanouchi *et al.* [34] for details.

(d) Kinetic proofreading in QS signal recognition. Sequential reactions involving signal (A) binding to an R-protein (R) and dimerization of R-A complex constitute a mechanism analogous to the canonical Hopfield-Ninio model of kinetic proofreading (gray shade). Stabilization of R by A provides another layer of kinetic proofreading especially when R is unstable (red shade). Signal A could be cognate or non-cognate, the difference lying in the reaction rates of the steps shown. Redrawn from ref. [36].

Fig. 2. Synthetic systems with communication within a population

(a) A population controller. The circuit uses the schematic in Fig. 1a but here the signal-bound LuxR drives expression of a toxin CcdB. Redrawn from ref. [46]

(b) Density dependent invasion circuit. The circuit architecture differs slightly from (a) with the invasin gene (*inv*) expression controlled by communication. Redrawn from ref. [65]

(a)

(b)

(c)

Microbial consensus consortium

(d)

Predator

Prey

(e)

(f)

Fig. 3. Synthetic systems with communication between populations

(a) Pattern formation using signal senders and receivers (top left) with spatial signal gradient around senders (Bottom left). Receivers (top center) are programmed to express fluorescence (green curve) in a narrow range of signal concentrations (bottom center). The positioning of the detection band can be changed (dotted green) by manipulating the receiver circuit elements. Active parts of receiver circuit logic at different AHL concentrations are shown (Top right). In a circular domain with senders in the middle and receivers everywhere on the surface of the circle, a ring like pattern will emerge. Redrawn from ref. [55]

(b) Synthetic cooperative system. Cooperators constitutively synthesize the AHL signal C_4HSL via *RhlI*. Non-cooperators do not express *RhlI*. Both populations constitutively express *RhlR* which, when signal-bound, induces the expression of a chloramphenicol resistance gene (*catLVA*). Redrawn from ref. [76]

(c) Microbial consensus consortium (MCC). *LasI* and *RhlI* catalyze the synthesis of AHLs, $3OC_{12}HSL$ (red circles) and C_4HSL (green circles), which activate *LasR* and *RhlR*, respectively. The activated regulators then activate their target reporters, RFP and GFP respectively. Both populations need to be at sufficient density (not equal) for concurrent expression of both reporters. Redrawn from ref. [53]

(d) Synthetic predator-prey system. Predator and prey synthesize $3OC_{12}HSL$ (green circles) and $3OC_6HSL$ (red circles) via *LasI* and *LuxI*, respectively. Toxin, *CcdB*, is produced constitutively in the predator but is under P_{luxI} control in the prey. In the predator, antidote *CcdA* is under P_{luxI} control. $3OC_{12}HSL$ -bound *LasR* does induce expression from P_{luxI} . The predator requires sufficient number of prey cells to sustain enough expression of *CcdA* to survive. On the other hand, the prey dies due to *CcdB* expression when the density of predator is high. Redrawn from ref. [80]

(e) Another design of synthetic predator-prey system. The system consists of wild-type *E. coli* cells (predator) and Chinese hamster ovary cells (prey) in a medium containing ampicillin. Rapid growth of the predator limits the prey's growth by depleting nutrients. Prey constitutively expresses *BLA* that degrades ampicillin. Redrawn from ref. [82]

(e) A synthetic obligatory cooperation circuit with two mutually dependent yeast populations.

$X_{\rightarrow L}^{\leftarrow A}$ indicates yeast cell that overproduces lysine but requires external adenine. $Y_{\rightarrow A}^{\leftarrow L}$ overproduces adenine but requires external lysine supply. Redrawn from ref. [83]