

Research Article

Synthetic Crossfeeding Cocultures in Yeast: Computational Model of Autoregulation and Design of a Tryptophan Export Device

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In order to contribute to the design of crossfeeding systems, we modeled population control in a coculture of two crossfeeding strains of an organism, each of which secretes a metabolite the other strain requires to grow. Differential equations show that the steady-state population ratio can be tuned by varying the ratio of the metabolite secretion rates, as long as they fall within a range determined by the nature of the organism. Numerical simulations of Trp/His crossfeeding in budding yeast suggest that the time required to reach steady state populations critically depends on the capacity of the cells to uptake the crossfeeding amino acids. We also engineered and evaluated a novel genetic device that secretes tryptophan-rich peptides with a cell penetrating sequence. Experimental validation showed that the device increases tryptophan secretion and enables growth of a *trp*⁻ strain in a coculture in synthetic medium lacking tryptophan.

1. Introduction

The complexity of synthetic biology devices can be increased by physically isolating subsystems in different cells, creating a “division of labor” [1]. Besides reducing the load on each cell, this scheme allows reutilizing the same components

in different strains for different functions. For this scheme to work robustly it is desirable that the different strains in the culture maintain a fixed proportion. Synthetic biology devices with controlled populations of multiple cell types have been engineered through crossfeeding, in which each strain in a coculture secretes a metabolite the other strain

needs to take from the medium in order to grow [2, 3]. However, population control through crossfeeding is not a mature technique and there is plenty of space for innovations.

The design of crossfeeding devices involves understanding the range of conditions for autoregulated growth and the timeframe required to reach steady state populations [1–4]. Previous models accounted for cell proliferation, death, and crossfeeding [2–4]. Here we present a simple model of crossfeeding and study the conditions under which population control can be achieved. The model includes a limited carrying capacity that accounts for growth saturation of the culture and a Hill exponent in the Monod equation for culture growth [3, 4] to account for the observed behavior of our yeast strains (See Supplementary Material available online at <http://dx.doi.org/10.1155/2015/178514>). Additionally, we present a designed genetic device that can be used in the implementation of a crossfeeding system. We engineered and validated a device that secretes tryptophan-rich peptides. Furthermore, a cell-penetrating peptide upstream of the “payload” was introduced to enhance peptide uptake.

2. Materials and Methods

2.1. Modeling. We used ordinary differential equations (ODEs) to model the strains population dynamic and amino acids concentrations as explained in the Results section. To analyze the steady state (SS) solution of the system we equaled all ODEs to zero and solved the nonlinear algebraic system using the software Mathematica (Wolfram Research). We assessed as “biologically relevant” the solutions that resulted in meaningful results (e.g., positive populations) for parameters values within physiological ranges. Numerical simulations were done using package deSolve for R and custom scripts.

2.2. BioBrick Sequence Design and Manipulation. Several of the BioBricks used in our designs are from organisms other than yeast. In order to optimize the expression of the corresponding constructs, we retrotranslated the designed amino acid sequences and optimized the DNA sequence using the R package GeneGA, which takes into account codon usage and messenger secondary structure. The devices were synthesized as gBlocks (Integrated DNA Technologies). Each gBlock was flanked at the 5′ end by an XhoI restriction site, followed by the BioBrick prefix and a BamHI restriction site. The device is followed at the 3′ end by a BioBrick suffix and a NcoI restriction site. The XhoI, BamHI, and NcoI sites enable directional cloning into the multiple cloning sites of the yeast plasmids used. They are also absent from the RFC10 BioBrick standard set. Two additional HindIII restriction sites flank the Trojan peptide sequence and allow selective removal (with a Lys-Leu scar). The final sequences are available at the BioBrick Registry, entries BBa_K792010 (Trp-export device I) and BBa_K792012 (Trp-export device II).

2.3. Sequence Manipulation and Cloning. Plasmid backbones pCM182 and gBlocks BBa_K792010 and BBa_K792012 were digested with BamHI and PstI restriction enzymes. The digested vectors were purified by agarose gels. Vectors and

devices were ligated overnight using T4 ligase. The ligation products were transformed into DH5 α *E. coli*, and the bacteria were plated on LB-agar with ampicillin and incubated overnight at 37°C. Colonies were then inoculated into liquid cultures (LB + ampicillin) and incubated overnight at 37°C. Plasmids minipreps were made. The presence of the inserts was checked by digestion with restriction enzymes and 1% agarose gel (1 kb and 100 bp ladders as markers).

2.4. Measurement of Tryptophan Secretion. We quantified tryptophan in the culture medium by measuring fluorescence intensity between 330 and 370 nm after excitation at 295 nm in an Aminco Bowman SLM2 spectrofluorometer. We calibrated the assay by measuring the fluorescence of serialized 1:2 dilutions of synthetic complete medium (SC, BSM formulations, BIO-101, Qbiogene, Irvine, CA, with 20 μ g/mL tryptophan) with SC medium lacking tryptophan (–T, BSM formulations, BIO-101, Qbiogene, Irvine, CA). Measurements on medium complemented with relevant concentrations of leucine, uracil, and histidine showed no changes in fluorescence intensity, indicating that our assay is specific for tryptophan.

Starters of each strain were grown to exponential phase in –T media, at 30°C with shaking. Cells were pelleted and washed with SC medium lacking tryptophan and histidine (–H–T, BSM formulations, BIO-101, Qbiogene, Irvine, CA). The cultures were grown at 30°C with shaking for 5 hours and the optical density at 600 nm was recorded every hour. The amount of tryptophan present in medium was quantified at the end of the experiment. Since the experiment was done during exponential growth, we expect cell lysis to be negligible and all tryptophan in the medium to come from the secreting device. As a control we grew the same strain transformed with an empty pCM182 plasmid.

2.5. Epifluorescence Microscopy. We used an Olympus IX-81 inverted epifluorescence microscope to quantify the population of each strain in the coculture. Aliquots (50 μ L) of each culture were placed in 384-well glass-bottom plates and left to settle for a few minutes. Three sets of brightfield, YFP (excitation 490–510 nm and emission 520–550 nm), CFP (excitation 426–446 nm and emission 460–500 nm), and RFP (excitation 530–585 nm and emission 610–675 nm) fluorescence images were acquired in each well. Images were segmented with Cell-ID and analyzed with the R package Rcell as explained in [5]. Individual cells were classified based on their YFP and CFP fluorescence level. Cells with low levels for both YFP and CFP fluorescence channels and cells with high fluorescence in the RFP channel were removed from the analysis, as these show dark nonretractile profiles in bright field images, indicative of cell death.

2.6. Coculture Experiments. For the coculture experiments starter cultures of each strain were grown to exponential phase in an adequate synthetic complete medium (e.g., –T for strains carrying pCM182). Cells were washed twice with –H–T medium and resuspended in 2 mL of –H–T medium supplemented with 100 μ g/mL ampicillin to avoid bacterial

contaminations. The initial cell density in each tube was set to $OD_{600} = 0.01$, which is approximately $3 \cdot 10^5$ cells/mL. Three biological replicates were done for each coculture condition. Tubes were incubated at 30°C with shaking and aliquots were taken every day for the length of the experiment and analyzed as detailed above. At the end of the experiment the OD_{600} of each tube was measured in a spectrophotometer. Statistical analysis was done using linear mixed-effects models (nlme package for R) for the epifluorescence microscopy data and Tukey's honest significant difference test for the optical density determinations (stats package for R).

3. Results and Discussion

3.1. A Model for Autoregulation of Synthetic Cocultures by Crossfeeding. We developed an ordinary differential equation model of a crossfeeding coculture taking into account four variables; N_x ($x = a, b$), the concentrations of two auxotrophic strains, in cells/mL, and AA_x the concentrations of two crossfeeding metabolites, in molecules/mL. We assume that (a) the medium can support a maximal density of cells, called carrying capacity or CC. (b) Each strain produces and secretes the crossfeeding metabolite at a constant rate p_x . (c) The number of molecules of AA_x required for a cell to duplicate is d_x . (d) Each cell uptakes only the metabolite it does not produce at a rate that depends only on the concentration of that metabolite. (e) The growth rate of each strain is a Hill function of the concentration of the metabolite it does not produce. K_x are the concentrations of metabolites at which half-maximal proliferation rates are obtained and k_{\max} is the strain-independent proliferation rate in complete medium. (f) Each cell has a fixed probability of dying per time interval D , regardless of the strain. (g) Metabolite release due to cell death is negligible. Accordingly, release of metabolites by cell death does not support crossfeeding at endogenous metabolite levels [2]. (h) The metabolite uptake rate does not depend on the culture's total cell density.

Based on these assumptions we wrote the following differential equations for the time evolution of each strain:

$$\begin{aligned} \dot{N}_a &= N_a \cdot \left(k_{\max} \frac{AA_a^n}{K_a^n + AA_a^n} \right) \cdot \left(1 - \frac{N_a + N_b}{CC} \right) - D \cdot N_a, \\ \dot{N}_b &= N_b \cdot \left(k_{\max} \frac{AA_b^m}{K_b^m + AA_b^m} \right) \cdot \left(1 - \frac{N_a + N_b}{CC} \right) - D \cdot N_b. \end{aligned} \quad (1)$$

The positive term in these equations accounts for cell division. It is the product of the total number of cells of the particular strain, the growth rate at the current concentration of the auxotrophic metabolite (modeled by a Hill function), and a factor that accounts for the limited carrying capacity of the culture, that is, the depletion of nutrients not explicitly modeled (e.g., glucose) and/or the accumulation of waste products that inhibit growth (e.g., ethanol). The negative

term in these equations represents cell death. The metabolites dynamic are given by the following equations:

$$\begin{aligned} \dot{AA}_a &= p_a \cdot N_b - d_a \cdot N_a \cdot \left(k_{\max} \frac{AA_a^n}{K_a^n + AA_a^n} \right), \\ \dot{AA}_b &= p_b \cdot N_a - d_b \cdot N_b \cdot \left(k_{\max} \frac{AA_b^m}{K_b^m + AA_b^m} \right). \end{aligned} \quad (2)$$

The positive term captures the secretion of AA_x by the metabolite producing strain (N_a secretes AA_b and vice versa), and the negative term considers the consumption of AA_x required to make new cells. This last term is the amount of AA_x required for the cells to duplicate d_x , times the division rate of the metabolite consuming strain. The factor accounting for carrying capacity is not included, which means that in our model cells in nearly saturated cultures ($N_a + N_b \sim CC$) still uptake the metabolite they do not produce.

We found two biologically relevant steady state solutions for the system (see Section 2.1). In the first solution the total cell population $N_t = N_a + N_b$ is zero, standing for a culture that dies out because one strain is missing or the initial density is too low. The second solution represents a culture that can thrive and is given by the following equations:

$$N_t = CC \cdot \left(1 - D \cdot \sqrt{\frac{d_a d_b}{p_a p_b}} \right), \quad (3)$$

$$X_a = \frac{1}{1 + \sqrt{p_b d_a / p_a d_b}}, \quad (4)$$

$$\begin{aligned} AA_a &= K_a \left(k_{\max} \sqrt{\frac{d_a d_b}{p_a p_b}} - 1 \right)^{-1/n}; \\ AA_b &= K_b \left(k_{\max} \sqrt{\frac{d_a d_b}{p_a p_b}} - 1 \right)^{-1/m}. \end{aligned} \quad (5)$$

Note that the fraction of strain a in the community $X_a = N_a/N_t$ is independent of initial conditions; that is, the system autoregulates as intended (4), and X_a can be tuned by engineering the secretion rates of the crossfeeding metabolites (Figure 1(a)).

The culture dies out if $(p_a/D) \cdot (p_b/D) < d_a \cdot d_b$ (3) (Figure 1(b), Region I). This condition is satisfied if the total metabolite secretion during the life span of a cell does not suffice to build at least one new cell, for both relevant metabolites ($p_x/D > d_x$ for $x = a, b$). Note however that a higher secretion rate for one metabolite can still result in a culture that dies out if the secretion rate of the other metabolite is low enough. The total steady state concentration of cells $N_t = N_a + N_b$ increases with secretion rate above this limit and asymptotically approaches the carrying capacity of the culture (Figure 1(b), Regions II and III).

The medium saturates with the crossfeeding metabolites if $(p_a/k_{\max}) \cdot (p_b/k_{\max}) > d_a \cdot d_b$ (5). This occurs if the amount of metabolites secreted in the characteristic time $\tau_c = 1/k_{\max}$ ($\tau_c = \tau_d/\ln(2)$ where τ_d is the doubling time

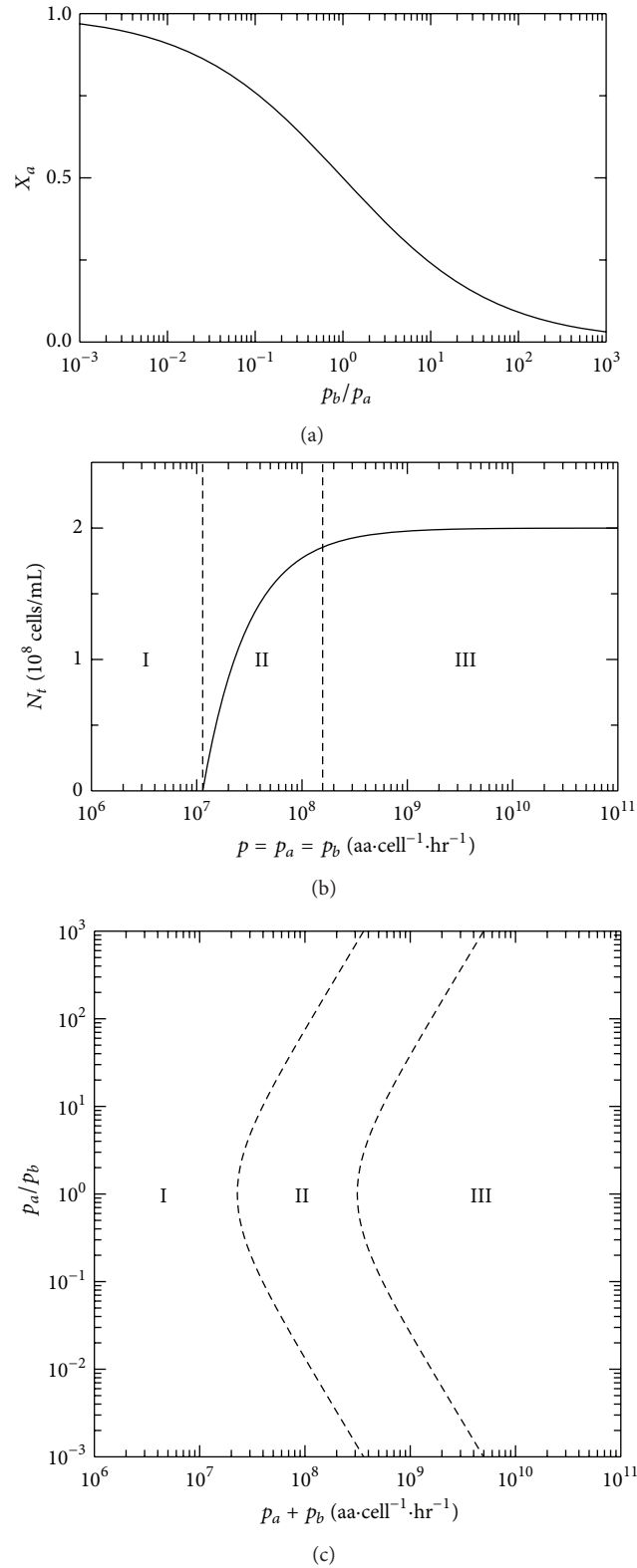


FIGURE 1: Steady state behavior of the crossfeeding model. (a) Fractional population of strain a as a function of the ratio of the two metabolite secretion rates for $d_a = d_b$. (b) Number of cells in the coculture as a function of the secretion rate. (c) Regions I, II, and III for different values of p_a/p_b and $p_a + p_b$. The model parameters used were $CC = 2 \cdot 10^8$ cells/mL [7], $D = 0.029 \text{ hr}^{-1}$ [8], $d_a = d_b = 3.93 \cdot 10^8$ amino acids/cell [6, 7], and $k_{\max} = 0.40 \text{ hr}^{-1}$ [6, 7].

of an exponential culture in complete medium) exceeds the amounts required for each strain to duplicate. Such a culture (Figure 1(b), Region III) would grow in a regulated manner until the total secretion of crossfeeding metabolites surpasses the total consumption. Beyond this point, the system will not return to the intended population ratio after a perturbation. As before, a lower secretion rate for one metabolite can still result in an unregulated culture if the secretion of the other is high enough. If $(p_a/k_{\max}) \cdot (p_b/k_{\max}) < d_a \cdot d_b$, the system can autoregulate itself (Figure 1(b), Region II).

Combining the growth and autoregulation conditions we obtain $D \cdot \sqrt{d_a d_b} < \sqrt{p_a p_b} < k_{\max} \cdot \sqrt{d_a d_b}$ (Figure 1(b), Region II). For a given organism D , k_{\max} , d_a , and d_b are generic values that are not easily modified. On the other hand, the secretion rates p_a and p_b can be seen as tunable parameters that must lie within a defined range. Too low and the culture dies out, too high and it gets out of control. Interestingly, changes in p_a and p_b can compensate each other.

Region II is located at different values of the total secretion rate $p_a + p_b$ for different ratios of the secretion rates p_a/p_b (Figure 1(c)). Therefore, not only the ratio of the secretion rates but also their sum must be taken into account to tune the microbial community to different X_a .

3.2. Application to Trp/His Crossfeeding in Budding Yeast. Population control in cocultures of model organisms could in principle make use of well characterized auxotrophies. We applied our model to Trp/His crossfeeding in *Saccharomyces cerevisiae*, using numerical simulations and physiological values for the model parameters. The growth rate of yeast in complete medium k_{\max} is 0.40 hr^{-1} [6, 7], and the death rate D is 0.029 hr^{-1} [8]. The carrying capacity of a yeast culture CC is approximately $2 \cdot 10^8$ cells/mL [7]. The amounts of histidine and tryptophan in a yeast cell are $d_{\text{His}} = 5.82 \cdot 10^8$ amino acids/cell and $d_{\text{Trp}} = 2.65 \cdot 10^8$ amino acids/cell [6, 7].

According to these estimations and to our model, the amino acid secretion rates must satisfy $\sqrt{p_a p_b} \in [1.1 \cdot 10^7; 1.6 \cdot 10^8]$ amino acids \cdot cell $^{-1}\cdot$ hr $^{-1}$ in order for the system to grow in a regulated manner to the intended population ratio (Region II). To implement the secretion of crossfeeding amino acids, we decided to engineer the cells to secrete Trp rich peptides (see below). Therefore, the maximum secretion rate is likely to be limited by the cell's capacity to synthesize and export peptides. We estimated the peptide secretion capacity of yeast cells in two ways, first, based on the total protein synthesis capacity of yeast cells, estimated in $2 \cdot 10^{10}$ protein elongation events per hour [9]. If 1% of this capacity is used to produce secretion peptides, we obtain a secretion rate of $2 \cdot 10^8$ amino acids \cdot cell $^{-1}\cdot$ hr $^{-1}$. As an independent estimation we used the reported secretion rate of the α -factor mating pheromone peptide, $2.6 \cdot 10^7$ amino acids \cdot cell $^{-1}\cdot$ hr $^{-1}$ [10]. Thus, the requirement of the system is compatible with the secretion capacity of yeast cells.

In order to perform numerical simulations of cross-feeding in yeast, we determined the K_x values and Hill exponents for two yeast strains auxotrophic for Trp and His, obtaining $K_{\text{Trp}} = 1.17 \pm 0.04 \cdot 10^{16}$ amino acids/mL, $n_{\text{Trp}} = 1.98 \pm 0.11$, $K_{\text{His}} = 2.98 \pm 0.29 \cdot 10^{16}$ amino acids/mL,

and $n_{\text{His}} = 1.44 \pm 0.10$ (See Supplementary material). A Region II coculture is able to grow and reach a fixed strain proportion, at a rate slower than k_{\max} (Figures 2(a) and 2(b)). Interestingly, the lag time for cell proliferation matches the time required for the crossfeeding amino acids to accumulate to their correspondent K_x concentrations. Furthermore, we found shorter lag times not only for higher initial cell density, but also for lower values of K_x (Figure 2(c)). The lag time becomes infinite for small initial inoculums and/or high values for K_x (Figure 2(c)), because even though the amount of amino acid secreted in the life of a cell is enough to make several daughter cells (i.e., parameters in Region II), the concentration of amino acid does not reach levels that allow the cells to incorporate them (See Supplementary material for details).

Our model thus suggests that Trp/His crossfeeding in yeast is in principle feasible and may be tuned by varying the effective concentrations K_x of the crossfeeding amino acids in the medium.

3.3. Design and Construction of a Tryptophan-Secreting Device. We engineered a device for amino acid secretion in budding yeast. The conceptual structure of the device consists of the parts shown in (Figure 3), listed from 5' to 3'.

First, we used a Kozak sequence to promote the initiation of translation. We used BioBrick BBa_J63003, a yeast Kozak sequence in the Registry of Standard Biological Parts [11], and contributed BBa_K792001 Kozak sequence from the 5' UTR of the yeast MF α 1 (α -factor mating pheromone gene). Second, we selected a signal peptide that would target the product of the gene for secretion. We used BioBrick BBa_K416003 from the Registry and contributed BBa_K792002, a secretion tag from yeast MF α 1. This last module is likely to work well when combined with the Kozak sequence from the same gene BBa_K792001, as the natural 5' end of the MF α 1 transcript is reconstituted. Third, we included a Trojan peptide reported to increase the internalization rate of the peptide without the need for a specific receptor in the target cell [12]. The intended effect was to increase the apparent affinity of the target cell for the crossfeeding amino acid and thereby decreasing the lag time for the growth of a crossfeeding coculture. We used the natural HIV TAT penetratin TATYGRKKRRQRRR (BBa_K792003) and the designed sequence R $_{11}$ (Polyarginine, BBa_K792004) [13]. Fourth, we selected a peptide "payload" rich in tryptophan and designed a novel one. We reasoned that the payload should be rich in tryptophan in order to ensure effective crossfeeding, but a tryptophan-only payload will be aggregation-prone and show low solubility. TrpZipper2 is the soluble, monomeric, and beta hairpin-forming sequence SWTWENGKWTWK [14] (BBa_K792006), and PolyW $_b$ is the novel sequence (WGDWDGWGKWKG) $_3$ (BBa_K792008). PolyW $_b$ includes glycine residues for flexibility and charged aspartate and lysine residues for solubility. We also avoided repeating the same codon in tandem to minimize local tRNA depletion and avoid repetitive DNA sequences.

We combined these parts into two different devices which were submitted to the Registry of Standard Biological Parts [11]: BBa_K792010, composed of MF α 1 Kozak sequence

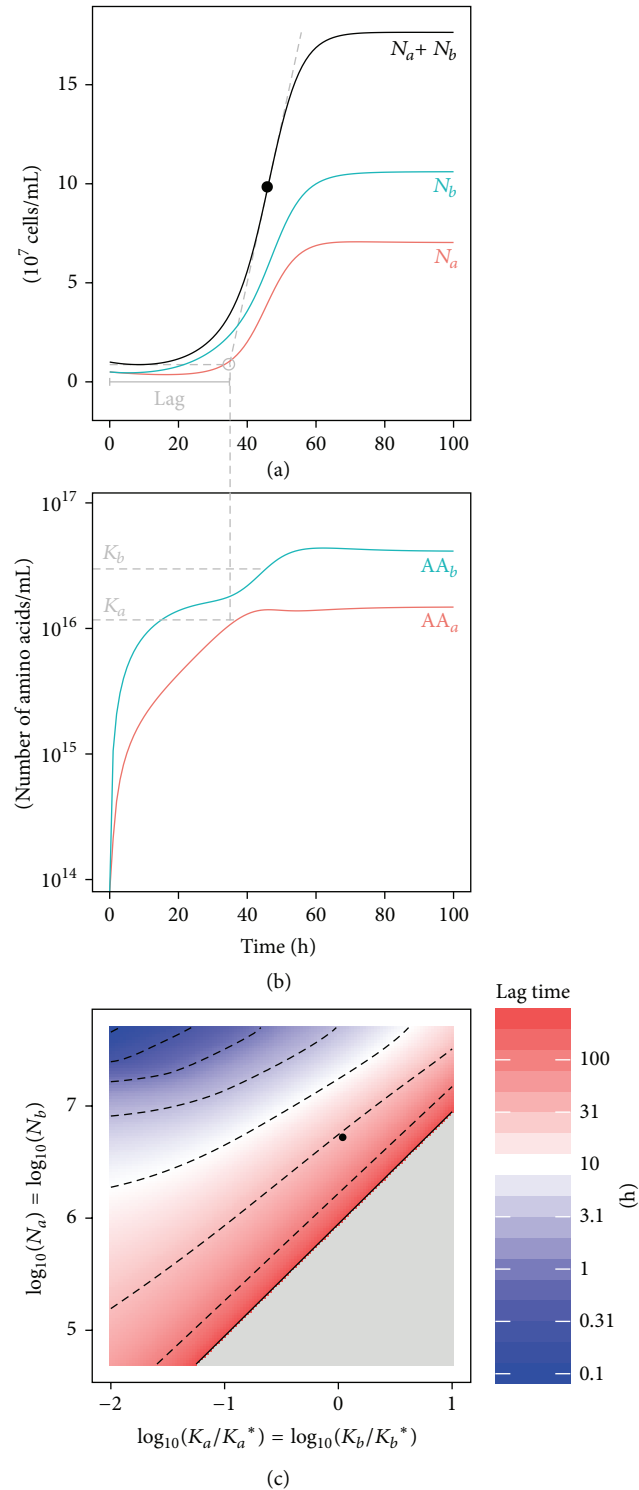


FIGURE 2: Dynamics of the crossfeeding model. Numerical simulation of a coculture seeded with $5 \cdot 10^6$ cells/mL of each strain. p_a and p_b are $0.43 \cdot 10^8$ and $2.16 \cdot 10^8$ amino acids \cdot cell $^{-1}\cdot$ hr $^{-1}$, respectively. See text for values of the model parameters. (a) Time evolution of the strains. Lag time was operationally defined as the time at which the line tangent to the inflexion point of the growth curve intersects the horizontal line of the basal level of the culture (grey dashed lines). (b) Amino acid concentrations. Horizontal dashed grey lines mark the values of K_a and K_b , respectively, and the vertical dashed line the lag time as defined in (a). (c) Growth lag time in hours as a function of relative K_x (normalized to the experimental parameter K_x^* , x-axis) and initial cell populations (y-axis). The dashed black lines are contour curves of value 0.1, 0.31, 1, 3.1, 10, 31, and 100 hours. The grey region corresponds to cultures with no growth after 300 hours. The thick black line is the boundary defined in the supplementary material. The black point represents the parameters used in (a) and (b).

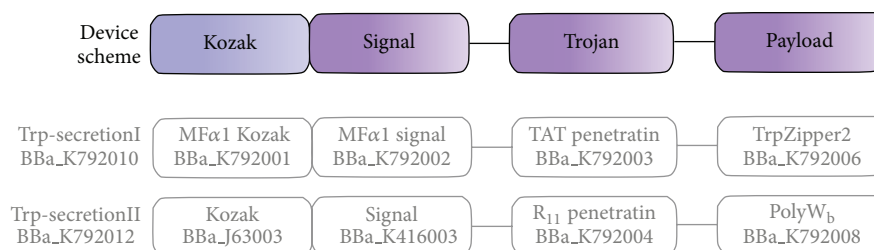


FIGURE 3: BioBrick design. Scheme of DNA structure for the devices with their constitutive parts. Untranslated Kozak region is colored blue while translated sequences are colored violet. Note that only the two modules on the right reach the fed cells.

and secretion tag, TAT penetratin, and TrpZipper2 payload. BBa_K792012 is composed of BBa_J63003 Kozak sequence, BBa_K416003 secretion tag, R₁₁ penetratin, and PolyW_b payload. The modular design and the presence of several restriction sites allow a straightforward reshuffling of parts in the future.

We cloned the two tryptophan-secreting devices into pCM182 yeast expression plasmid. This plasmid has a TRP1 selection marker and a tetracycline repressible promoter upstream of the multiple cloning sites. We transformed pCM182 with and without the BBa_K792010/12 devices into strain TCY3081 (*trp1 his3 YFP*). Note that these plasmids confer both the ability to produce and secrete Trp to any $\Delta trp1$ yeast strain. We called the resulting strains “H⁻” (harboring empty pCM182), “H⁻ Trp-secretionI” (harboring pCM182_BBa_K792010), and “H⁻ Trp-secretionII” (harboring pCM182_BBa_K792012). The H⁻ notation denotes that these strains are auxotroph for histidine.

3.4. Measurement of Tryptophan Secretion. We took advantage of the fluorescent properties of tryptophan to measure the secretion of tryptophan-rich payloads, for the engineered H⁻ Trp-secretionI and H⁻ Trp-secretionII strains. We set up a spectrophotometric assay to quantify the amount of secreted tryptophan in the yeast culture medium (see Section 2.4). Briefly, we measured fluorescence emission spectra between 330 and 370 nm exciting at 295 nm. Representative spectra of the calibration curve are shown in Figure 4 as grey lines.

We inoculated -T medium with the H⁻ Trp-secretionI and H⁻ Trp-secretionII strains, at an initial OD₆₀₀ of 0.1. We followed exponential culture growth by measuring OD every hour until an OD₆₀₀ of 0.8 was reached in approximately five hours. We measured tryptophan concentration in each culture using our spectrophotometric assay (red, green, and cyan lines in Figure 4). Tryptophan in the medium may originate by both, payload secretion due to our engineered device and preexisting mechanisms such as cell lysis and passive amino acid diffusion. Tryptophan concentration in a culture of the designed tryptophan-secreting strains was about ten times higher than in a culture of the same strain transformed with an empty plasmid.

3.5. Feeding Assay. We chose the H⁻ Trp-secretionI strain for experimental validation as tryptophan provider due to its higher export activity (Figure 4). We performed

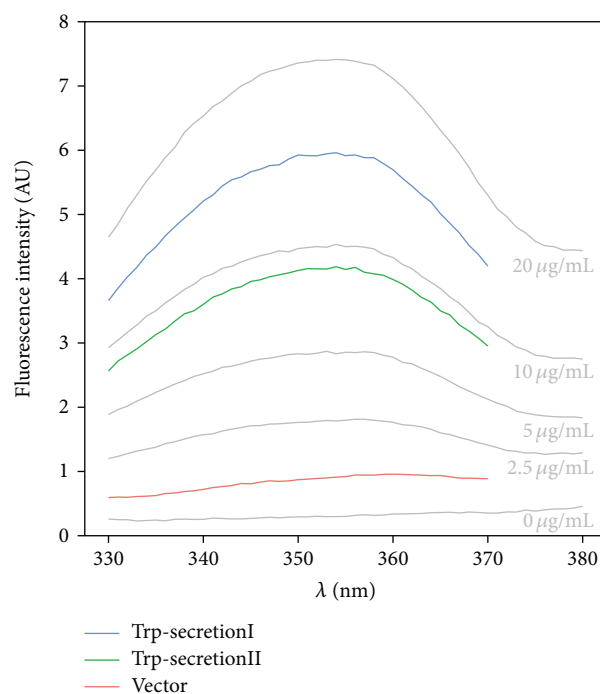


FIGURE 4: Determination of tryptophan secretion for the engineered Trp-secretion I and Trp-secretion II strains. Fluorescence emission spectra (excitation at 295 nm) of -T culture media. Grey lines: -T medium with different added concentrations of tryptophan. Blue line: -T medium inoculated with H⁻ Trp-secretionI strain. Green line: -T medium inoculated with H⁻ Trp-secretionII strain. Red line: -T medium inoculated with a yeast carrying empty pCM182 plasmid.

a coculture experiment with a tryptophan auxotrophic strain (T⁻ YAG3905, *trp1 HIS3 CFP*) as the tryptophan consumer. The T⁻ strain expresses cyan fluorescent protein (CFP) and the H⁻ strain expresses yellow fluorescent protein (YFP) to allow for quantification of the strains proportion in the culture. We quantified the concentration of the strain or strains in the culture using epifluorescence microscopy (see Section 2.5 for details). The results are shown in Figure 5. Pure cultures do not show growth in the absence of the amino acids required, as expected from the strain’s auxotrophies (Figures 5(a) and 5(b)). Next we coculture pairs of strains that have not been engineered to crossfeed, shown in Figure 5(c).

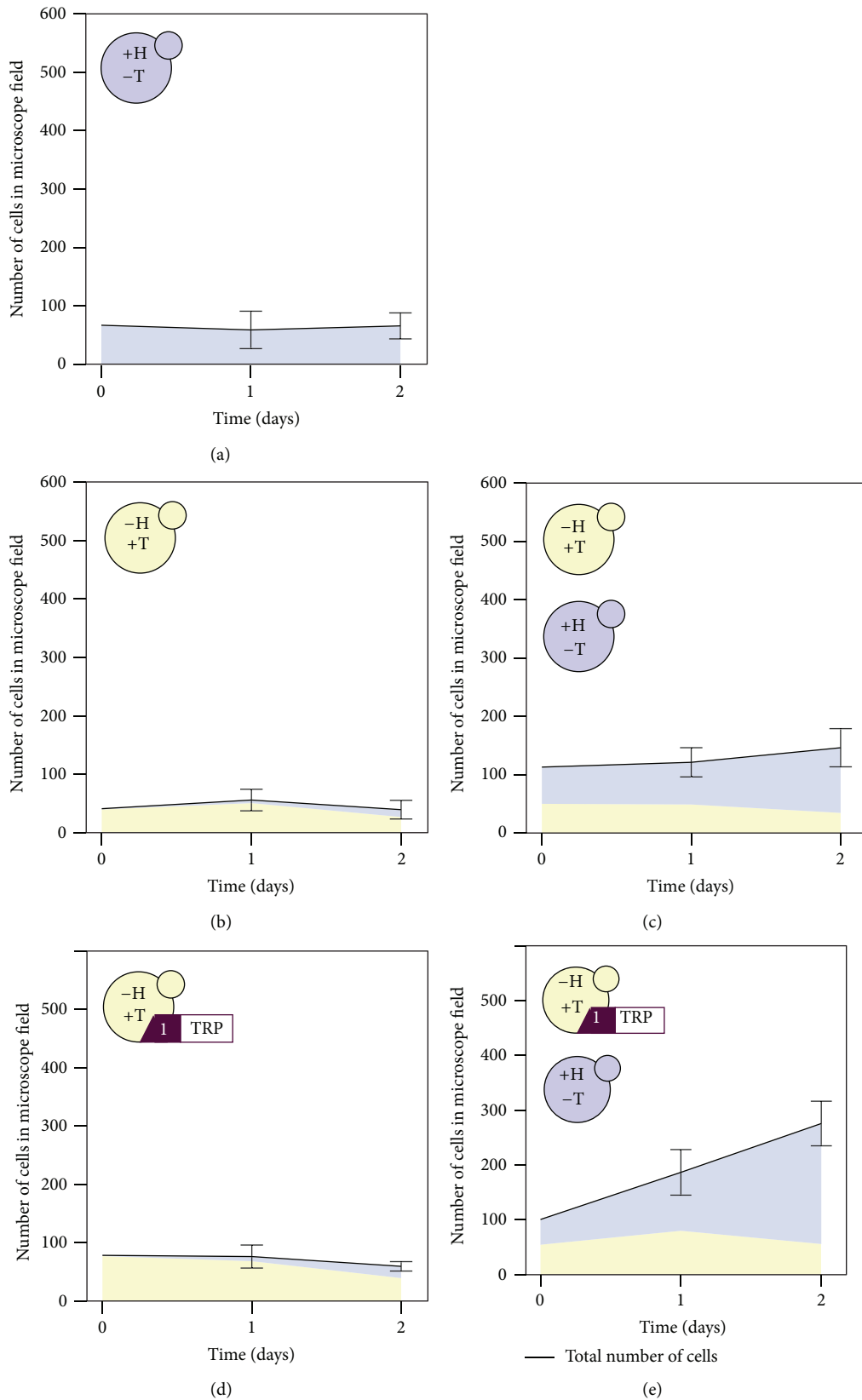


FIGURE 5: Culture growth of the following strains or pairs thereof. The bold line indicates the total number of cells, while the yellow and blue areas indicate the proportion of H^- and T^- cells, respectively. The error bars indicate the standard deviation of three replicas of the experiment. H^- and H^- Trp-SecretionI cells are shown in yellow, and the T^- cells (fed population) are shown in cyan. Panels correspond to cultures of the following strains: (a) T^- ; (b) H^- ; (c) T^- and H^- ; (d) H^- Trp-secretionI; (e) H^- Trp-secretionI and T^- .

We observed no significant growth ($P = 0.12$, linear mixed-effects model), indicating that basal crossfeeding via cell lysis and/or passive amino acid diffusion is negligible. On the other hand, Figure 5(e) shows that the H^- Trp-SecretionI/ T^- coculture does display significant growth of the T^- strain ($P < 10^{-4}$, linear mixed-effects model). This is evidence that our secretion device works enhancing Trp-auxotroph strain growth by external feeding.

Quantifying culture growth using optical density at 600 nm yielded consistent results (See Supplementary material). The culture in the feeding assay reached a final OD_{600} of 0.18 ± 0.02 , which is relatively low when compared to the OD_{600} of 4.90 ± 0.03 reached by an overnight culture with saturating amounts of free Trp ($20 \mu\text{g/mL}$, See Supplementary materials). Nevertheless it is a conspicuous growth if we take into account that the culture was inoculated at an OD_{600} of 0.01, and that the H^- Trp-SecretionI strain did not show significant growth (Figure 5(e)).

4. Conclusions

Our model indicates that a crossfeeding coculture can attain autoregulated growth if the metabolite secretion rates lie within a region defined by the organism growth/death rates and the requirements for each metabolite. In order to tune the populations of the two strains, the ratio of the secretion rates and their sum must both be taken into account. Our calculation also suggests that Trp/His crossfeeding in yeast is in principle feasible, with equilibration times that depend on the efficiency of amino acid uptake.

We have engineered and validated two devices for tryptophan secretion in yeast. Our modular implementation follows the BioBrick framework for device standardization so that future designs can be optimized efficiently. The secretion rate is in the range required for regulated growth of the coculture. Yeast strains auxotrophic for tryptophan show growth only in the presence of a feeding strain with the device. This work is a step towards the design of stable and tunable cocultures of genetically engineered machines.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

All iGEM 2012 Buenos Aires team members conceived the project. Instructors Alejandro Daniel Nadra and Ignacio Enrique Sánchez coordinated the project and designed the devices. Instructors Alejandro Daniel Nadra, Ignacio Enrique Sánchez and graduated advisor Alan Bush wrote the paper. Undergraduate student Verónica Parasco designed and performed the modeling under the supervision of graduate advisor Alan Bush. Undergraduate students Manuel Giménez, Luciano Gastón Morosi, María Alejandra Parreño and Mario Rugiero designed and performed wet lab work under the supervision of graduate advisors Alan Bush, Germán Sabio

and Alicia Grande. Non-team member Alejandro Colman-Lerner provided the team with reagents, lab space, and expertise in budding yeast.

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