

Designing microbial consortia with defined social interactions

Wentao Kong^{1,2}, David R. Meldgin^{2,3}, James J. Collins^{4,5,6*} and Ting Lu^{1,2,3,7*}

Designer microbial consortia are an emerging frontier in synthetic biology that enable versatile microbiome engineering. However, the utilization of such consortia is hindered by our limited capacity in rapidly creating ecosystems with desired dynamics. Here we present the development of synthetic communities through social interaction engineering that combines modular pathway reconfiguration with model creation. Specifically, we created six two-strain consortia, each possessing a unique mode of interaction, including commensalism, amensalism, neutralism, cooperation, competition and predation. These consortia follow distinct population dynamics with characteristics determined by the underlying interaction modes. We showed that models derived from two-strain consortia can be used to design three- and four-strain ecosystems with predictable behaviors and further extended to provide insights into community dynamics in space. This work sheds light on the organization of interacting microbial species and provides a systematic framework—social interaction programming—to guide the development of synthetic ecosystems for diverse purposes.

Designer microbial consortia are communities of rationally designed, interacting microorganisms that are capable of producing desired behaviors^{1–4}. In the past decade, an array of such synthetic systems were developed, enabling various applications such as generation of specific ecological dynamics^{5–9}, promotion of species growth^{10,11} and synthesis of valuable chemicals^{12,13}. Compared to engineered isogenic populations, synthetic communities offer an increased degree of robustness for designed cellular functions and an expanded spectrum of functional programmability for complex tasks, thereby enabling novel and versatile biotechnological applications in complex settings¹. Synthetic microbial consortia have also emerged as a promising engineering tool to manipulate microbiomes, such as those in the human body and in the rhizosphere, which help to realize the enormous potential of microbiomes for therapeutic, environmental and agricultural purposes^{14–17}. However, despite increasing exciting proof-of-concept demonstrations, the utilization of such synthetic ecosystems is hampered by our limited ability in rapidly developing microbial ecosystems with desired temporal and spatial dynamics.

Cellular social interactions, such as competition and cooperation, are ubiquitous in microbial communities and are shown to be essential in specifying ecosystem dynamics¹⁸. For instance, engineering cross-feeding offers species co-existence^{10,19,20}, whereas building predator–prey interactions can lead to oscillatory, bistable or monostable behaviors⁵. Inspired by these findings, here we present a systematic framework for the design, construction and characterization of synthetic microbial communities, namely, social-interaction programming that combines modular pathway reconfiguration with model creation. Specifically, we employed a modular pathway reconfiguration approach to create six distinct consortia whose dynamics were specified by their underlying interaction modes. Using a modular approach similar to our experimental construction,

we also derived quantitative models that captured experimentally observed population patterns. We further showed that the models from two-strain consortia can be used to design and build three- and four-strain ecosystems with predictable behaviors and extended our investigations to yield insights into spatial community dynamics. Together, we established social-interaction engineering as an effective and valuable route for ecosystem programming.

Results

Modular pathway reconfiguration for programing social interactions. Synthetic gene circuits are typically constructed from the bottom up by assembling individual DNA parts^{21–26}; in principle, they can also be created through modular reconfiguration of existing gene clusters (Fig. 1a). Although it has barely been practiced, modular cluster reconfiguration can be powerful for rapid circuit engineering due to the increasing complexity of desired functionalities²⁷ and the high modularity of native gene networks²⁸. Inspired by this concept, we harnessed the modular biosynthesis pathways of nisin and lactococin A (lcnA) in *Lactococcus lactis* (Fig. 1b) to develop programmable cells that could form the basis of synthetic microbial consortia. Of note, nisin, an antimicrobial and quorum-sensing molecule, is encoded by an eleven-gene cluster involving five functional modules: precursor production, translocation and initial modification, secondary modification, immunity and signaling²⁹ (Fig. 1c); lcnA is an antimicrobial peptide whose underlying pathway consists of precursor production, translocation and immunity modules³⁰ (Fig. 1d).

The functional modularity of the pathways allowed us to rapidly generate different social interactions by selecting and tuning the molecules' signaling and bactericidal features through the alteration of module combinations. Specifically, we were able to create six synthetic microbial consortia that collectively enumerate all possible modes of pairwise social interactions^{18,31–33} (Fig. 1e).

¹Department of Bioengineering, University of Illinois at Urbana–Champaign, Urbana, IL, USA. ²Carl R. Woese Institute for Genomic Biology, University of Illinois at Urbana–Champaign, Urbana, IL, USA. ³Department of Physics, University of Illinois at Urbana–Champaign, Urbana, IL, USA. ⁴Institute for Medical Engineering and Science, Department of Biological Engineering, and Synthetic Biology Center, Massachusetts Institute of Technology, Cambridge, MA, USA. ⁵Broad Institute of MIT and Harvard, Cambridge, MA, USA. ⁶Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, MA, USA. ⁷Center for Biophysics and Quantitative Biology, University of Illinois at Urbana–Champaign, Urbana, IL, USA. *e-mail: jimjc@mit.edu; luting@illinois.edu

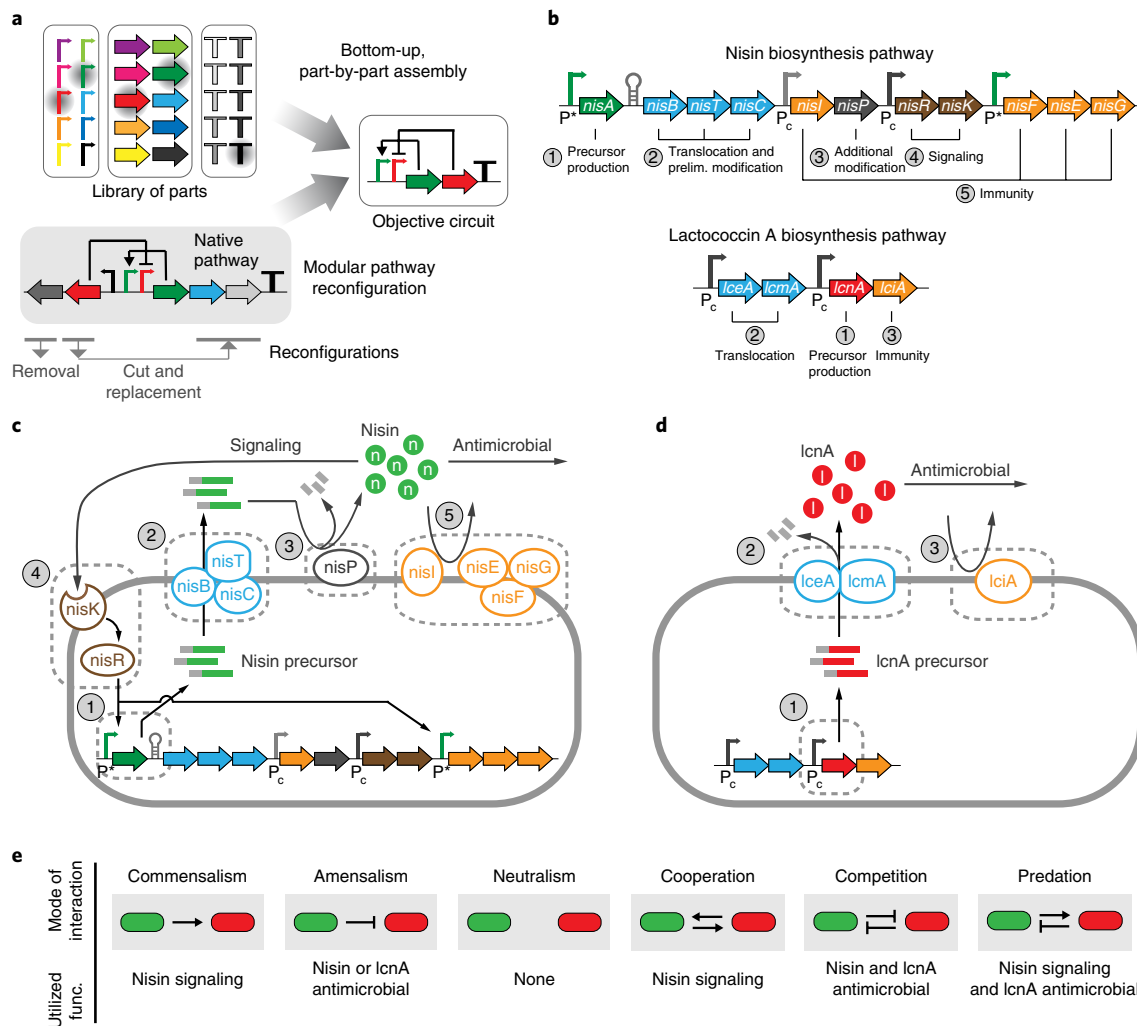


Fig. 1 | Modular pathway reconfiguration for engineering microbial consortia. **a**, Two approaches to engineering gene circuits. A circuit can be created by assembling selected genetic parts from scratch or through modular reconfiguration of existing gene clusters. **b**, Nisin and lactococcin A (lcnA) biosynthesis gene clusters. P* and Pc refer to nisin-inducible and constitutive promoters, respectively. **c**, Modular organization of the nisin pathway involves five functionally independent modules, including those for precursor production (1), translocation and initial modification (2), additional modification (3), signaling (4) and nisin immunity (5). **d**, Modular organization of the lcnA pathway contains three functionally independent modules, including those for precursor production (1), translocation (2) and lcnA immunity (3). **e**, Design of six two-strain consortia that differentially utilize the signaling and antimicrobial features of the bacteriocins.

Engineering consortia with unidirectional interactions. We started by constructing a two-strain consortium of commensalism in which one benefits the other. Using our previously developed synthetic biology platform^{34,35}, we generated one strain (CmA) by introducing into *L. lactis* (NZ9000) the full nisin pathway and the constitutively expressed tetracycline resistance gene (*tet^R*). We created the other strain (CmB) by transforming into NZ9000 a reconfigured version of the nisin pathway containing only the signaling and immunity modules as well as a nisin-inducible *tet^R* expression circuit (Fig. 2a; Supplementary Tables 1 and 2; Supplementary Fig. 1). Here, CmA was designed to secrete nisin to trigger the expression of *tet^R* in CmB, thereby conferring tetracycline resistance on CmB. Additionally, we inserted constitutively expressed fluorescent protein genes, *gfpuv* and *mCherry*, into the strains for observation and analysis.

As anticipated, CmA grew by itself in the GM17 media containing tetracycline because of its constitutive *tet^R* expression; in contrast, CmB by itself did not grow, as its resistance is not autonomous (Fig. 2b). However, CmA and CmB both grew when co-cultured

(1:1 ratio; Fig. 2b), suggesting that the presence of CmA conferred a growth benefit to CmB. Such behaviors were also observed in a variant of the consortium wherein the *tet^R* expression of CmA is nisin inducible (Supplementary Fig. 2a). Additional experiments showed that CmB grew in the tetracycline-containing media when nisin was supplemented (Supplementary Fig. 2b) but failed to grow when CmA's nisin production was abolished (Supplementary Fig. 2c). Furthermore, we showed that CmB growth was not due to tetracycline degradation or absorption by CmA (Supplementary Fig. 3a–e). Together, these results confirmed that the mechanism of commensalism is the tetracycline resistance of CmB induced by nisin released from CmA. Notably, as CmA and CmB were constructed from the same parental strain (NZ9000), they had an indirect nutrient competition during co-culture, and CmA had a reduced saturation density compared to that in monoculture.

We next engineered a consortium of amensalism, wherein one strain adversely affects the other by leveraging the bactericidal nature of nisin. Specifically, we created one strain (AmA) by transplanting into NZ9000 the full nisin pathway and the other strain

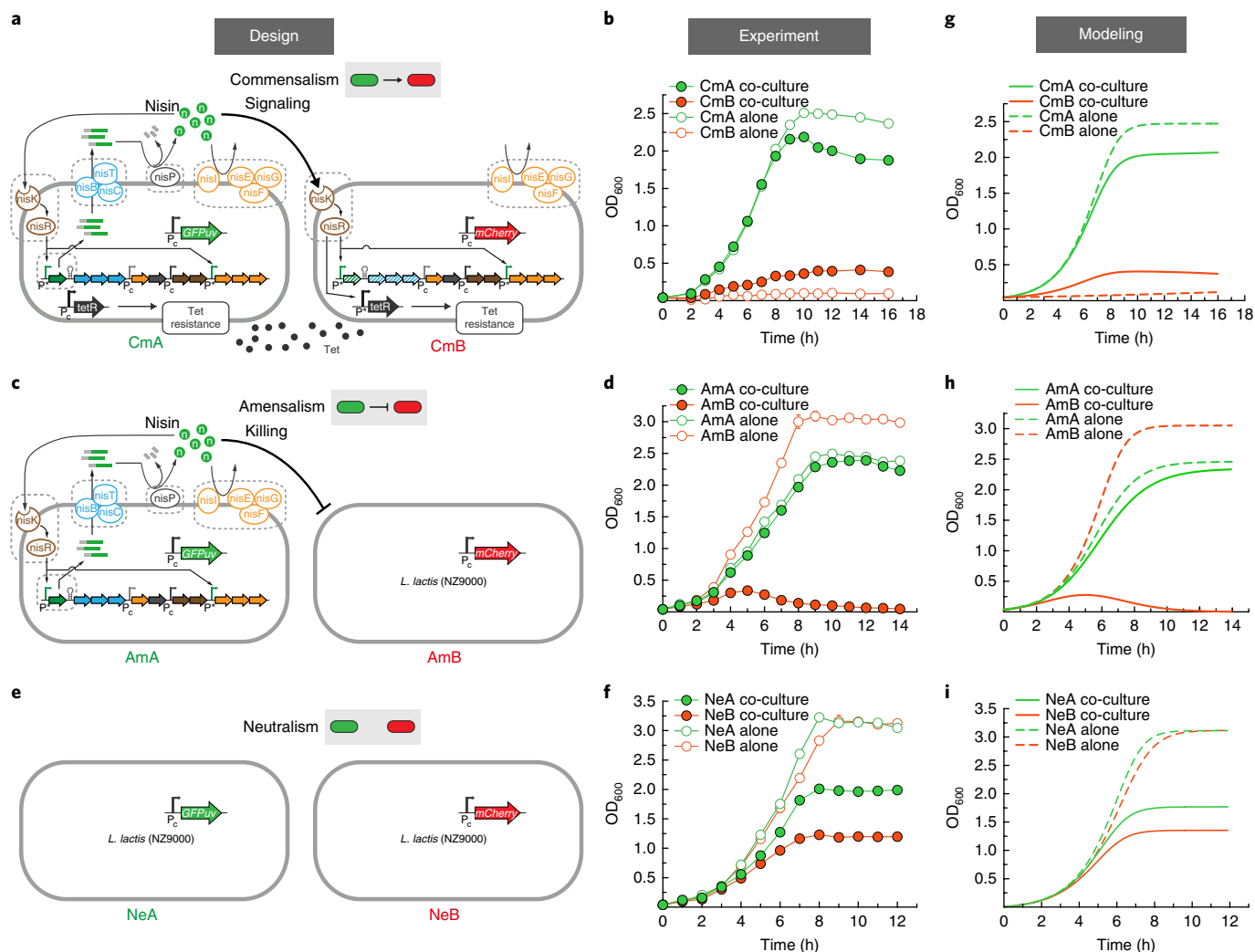


Fig. 2 | Synthetic consortia with one-way social interactions. **a**, Circuit diagram of a commensal microbial consortium. CmA is a nisin producer with constant tetracycline resistance. CmB has nisin-inducible tetracycline resistance. Here, genes filled with diagonal lines are knocked out. **b**, Growth of CmA and CmB of the commensal consortium, in monoculture and co-culture experiments using GM17 media supplemented with tetracycline. **c**, Circuit diagram of a consortium of amensalism. AmA is a nisin producer that inhibits the growth of AmB. **d**, Growth of AmA and AmB of the amensal consortium in monoculture and co-culture experiments. **e**, Circuit diagram of a neutral consortium, in which the two strains, NeA and NeB, do not have any active social interactions. **f**, Growth of two neutral strains, NeA and NeB, in monoculture and co-culture experiments. **g**, Simulated population dynamics for the commensal consortium. **h**, Simulated population dynamics for the consortium of amensalism. **i**, Simulated population dynamics for the neutral consortium. In panels **b**, **d** and **f**, closed and open circles stand for population growth in co-culture and monoculture experiments, respectively. In each co-culture experiment, strains were inoculated at 1:1 initial ratio. Experimental data are presented as mean (s.d.) from 3 independent experiments. In panels **g**, **h** and **i**, dashed lines correspond to monoculture growth, and solid lines correspond to co-culture growth.

(AmB) by simply introducing the vector pCCAM β 1 (Fig. 2c and Supplementary Fig. 1). Again, fluorescence protein genes (*gfpuv* and *mCherry*) were introduced as reporters. We found that, individually, AmA and AmB each grew up to saturation in GM17 media; however, when co-cultured, only AmA was able to grow (Fig. 2d), demonstrating the existence of a deleterious effect of AmA on AmB. Mechanistically, this effect arose from AmA's production of nisin, which inhibits the growth of AmB. Using the bacteriocidal nature of lcnA, we also constructed another version of amensalism by loading the lcnA pathway to NZ9000 to create a new toxin producer (AmA2) (Supplementary Figs. 4a and 1). Subsequent culturing experiments (Supplementary Fig. 4b) confirmed that the new ecosystem indeed involves a one-way deleterious interaction.

We further constructed a two-strain consortium of neutralism, as a control to commensalism and amensalism, by loading the vector

(pCCAM β 1) and reporters (*gfpuv* and *mCherry*; Fig. 2e and Supplementary Fig. 1) to NZ9000. The two resulting strains, NeA and NeB, were able to grow both individually and together (Fig. 2f), confirming that their social interaction is indeed neutral. Notably, the reduction of each strain's saturation density in co-culture was due to indirect nutrient competition.

To quantitatively describe the observed behaviors, we constructed a mathematical framework for two-strain community dynamics using ordinary differential equations (Methods; Supplementary Tables 3 and 4; Supplementary Note 1). The framework involves five variables: two for the strain populations, two for the interacting molecules produced by the strains and one for the nutrient in culture (see Eq. S1 in Supplementary Note 1). In concert with the modular configurability of the bacteriocin pathways for gene circuit development, this modeling framework allows modular

alterations to describe specific types of ecosystems. Upon data fitting (Supplementary Note 2), our derived models successfully captured the population-dynamics characteristics of the consortia of commensalism (Fig. 2g), amensalism (Fig. 2h and Supplementary Fig. 4c) and neutralism (Fig. 2i).

Developing consortia with bidirectional interactions. Leveraging the modularity of the nisin and *lcnA* pathways, we next created consortia involving two-way social interactions, namely, cooperation, competition and predation. Cooperation is the process in which multiple species work together to accomplish a task that yields mutual benefit. To create such a consortium, we designed the common task as nisin production, a multistep process that includes precursor production, translocation and post-translational modification (Fig. 1c). The task was divided by assigning one strain (CoA) for synthesizing and secreting nisin precursor and the other (CoB) for converting the precursor in the extracellular milieu into active nisin. Tetracycline resistance was chosen as the benefit for completing the task. We developed these two strains by modularly reconfiguring the nisin pathway and introducing nisin-inducible *tet^R* and reporter systems (Fig. 3a and Supplementary Fig. 1).

Our growth experiments showed that CoA and CoB did not grow in tetracycline-supplemented GM17 media unless they were co-cultured (Fig. 3b). For comparison, each of the strains was able to grow when the media was supplemented with nisin (Supplementary Fig. 5a). Additionally, abolishing the function of either strain (for example, precursor translocation by CoA and modification by CoB) resulted in no growth of either strain (Supplementary Fig. 5b,c). These results demonstrated the necessity of cooperation for the growth of the engineered strains.

Competition is an interaction between species in which the fitness of one is lowered by the presence of another. We designed an ecosystem with such an interaction using the antimicrobial features of nisin and *lcnA*. Experimentally, we generated two competing strains, named CpA and CpB, by introducing the nisin and *lcnA* pathways into NZ9000 separately (Fig. 3c and Supplementary Fig. 1). Notably, CpA and CpB are essentially the same as AmA and AmA2 (the two versions of toxin producers for amensalism), because competition is the superposition of two counter-oriented, detrimental interactions. We found that CpA and CpB both followed a logistic growth pattern individually, but when co-cultured, CpA grew with a substantial time delay (12 h) and CpB failed to grow (Fig. 3d), indicating that the fitness of both strains was reduced with their mutual presence. In this case, CpA won the competition and dominated the population. Notably, although CpB lost the contest, the *lcnA* it released at the beginning of the experiment caused the growth delay and reduction of CpA in co-culture.

It has been theoretically predicted that differential outcomes may arise in competition by altering ecosystem parameters such as relative interaction strengths³⁶. To test these predictions, we generated multiple variants of CpA and CpB by tuning their bacteriocin productivities, including CpA2 (a lowered translation initiation rate (TIR) of nisB), CpB2 (a wild-type TIR of *lcnA*) and CpB3 (an increased TIR of *lcnA*) (Supplementary Tables 1 and 2). Using different combinations of the variants, we indeed observed distinct competition outcomes including CpB dominance (Supplementary Fig. 6a) and close contests (Supplementary Fig. 6b,c).

Predation is a social interaction in which one species (prey) benefits another (predator) while being harmed. It is topologically equivalent to the combination of two unidirectional interactions, commensalism and amensalism, with opposite orientations. In light of this equivalence, we established a community of predation by assigning the strain CmA in the consortium of commensalism, a nisin producer with constitutive tetracycline resistance, as prey (PrA) and combining the circuits of CmB (nisin-inducible *tet^R*) and AmA2 (constitutive *lcnA* production) to form a predator (PrB; Fig. 3e

and Supplementary Fig. 1). In this design, PrA provides the benefit of tetracycline resistance to PrB by secreting nisin, whereas PrB hurts PrA through the release of *lcnA*.

When the resulting strains were co-cultured, PrA grew in the first 8 h but gradually declined to a steady density afterward, whereas PrB grew poorly at the beginning but faster later and eventually dominated the population, which was in contrast to the monocultures, wherein PrA grew normally but PrB failed to grow (Fig. 3f). The results confirmed the presence of the predatory relation between PrA and PrB, with the former serving as the prey and the latter as the predator. A variant of the ecosystem with similar behaviors involves the replacement of constitutive tetracycline resistance in PrA with nisin inducibility (Supplementary Fig. 7a). In addition, we confirmed that in this relationship both the beneficial and deleterious interactions are mandatory through the monoculture experiments with nisin supplementation (Supplementary Fig. 7b) and the co-culture experiments in which PrA or PrB was replaced by a neutral strain (Supplementary Fig. 7c,d).

To achieve a quantitative understanding of the above two-way ecosystems, we reconfigured our mathematical framework to create three ecosystem models (Supplementary Note 1 and Supplementary Table 5). Consistent with the experiments, these models were able to generate distinct community dynamics for the cases of cooperation (Fig. 3g), competition (Fig. 3h and Supplementary Fig. 6d–f) and predation (Fig. 3i). Together, the mathematical modeling and experimental development of synthetic ecosystems demonstrated that social-interaction engineering is a versatile and effective way to create desired two-strain microbial consortia.

Model-guided design of three- and four-strain ecosystems. To further illustrate the utility of our engineering method, we applied it to tackle a key challenge in microbiome and synthetic ecosystem research, namely, to design complex ecosystems with predictable dynamics. Specifically, we combined our established experimental consortia with mathematical models to determine whether the dynamics of complex communities (for example, three- and four-strain ecosystems) could be predicted from the behaviors of simple two-strain ecosystems. Using the models extended modularly from the two-strain cases (Supplementary Note 3), we first predicted the dynamic behaviors of eight three-strain microbial ecosystems, formed by introducing a third strain into the existing two-strain consortia. Importantly, in the process of model extension and dynamics prediction, all of the parameters from the two-strain communities remained fixed. The only new parameters were the growth rates of the newly introduced strains (i.e., the third strain), which had not been previously characterized.

To test the model predictions, we experimentally constructed eight third strains (CoAg, CoBg, CpAg, CpBg, CmAg, CmBg, AmAg and AmBg) in alignment with our models (Methods; Supplementary Tables 1 and 6; Supplementary Note 3). These third strains were derived from the corresponding parent strains in the two-strain consortia (CoA, CoB, CpA, CpB, CmA, CmB, AmA and AmB, respectively) using a colorimetric reporter gene (*gusA*) to substitute for the fluorescence reporter genes (*gfpuv* or *mCherry*). Therefore, the third strains have identical interaction modes to their ancestors but different growth rates because of the alteration of their reporter systems. For instance, CoAg has the same cooperation features as CoA but has a different growth.

After measuring the growth rates of the third strains, we mixed them with the two-strain consortia to form the eight three-strain communities and performed ecosystem culture experiments (Methods). Figure 4a–h shows the comparison of the model predictions (lines) and experimental measurements (circles) of the eight three-strain ecosystems, which demonstrates that the models successfully predicted the dynamics of the synthetic communities. Notably, the dynamics of these communities can be explained by

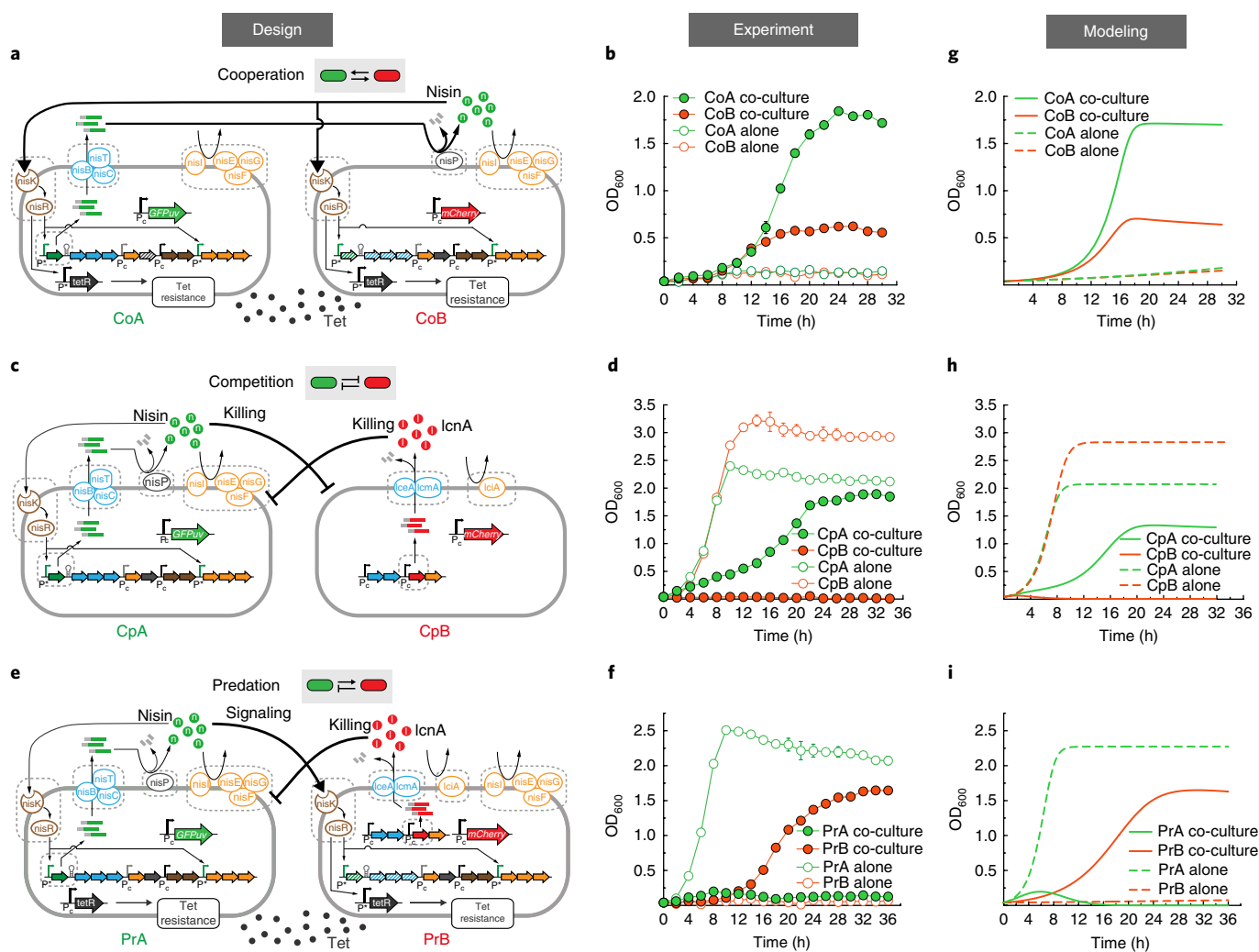


Fig. 3 | Synthetic consortia involving two-way social interactions. **a**, Circuit diagram of a cooperative consortium. CoA produces nisin precursor, and CoB modifies the precursor to produce active nisin, forming a cooperation of successful nisin production. Nisin induces the tetracycline resistance of both strains, which enables them to survive in tetracycline-supplemented media. Here, genes filled with diagonal lines are knocked out. **b**, Growth of two cooperative strains (CoA and CoB) in co-culture and monoculture experiments. **c**, Circuit diagram of a mutually competitive consortium. CpA is a nisin producer and CpB is an lcnA producer. **d**, Growth of two competitive strains (CpA and CpB) in co-culture and monoculture experiments. CpA outcompetes CpB in the co-culture experiment. **e**, Circuit diagram of a predatory consortium. PrA (prey) is a nisin producer with constant tetracycline resistance. PrB (predator) is an lcnA producer with nisin-inducible tetracycline resistance. PrA induces the growth of PrB by secreting nisin; in turn, PrB suppresses the growth of PrA by releasing lcnA. Here, genes filled with diagonal lines are knocked out. **f**, Growth of two predation strains, PrA and PrB, in co-culture and monoculture experiments. **g**, Simulated population dynamics for the cooperative consortium. **h**, Simulated population dynamics for the competing consortium. **i**, Simulated population dynamics for the consortium of predation. In panels **b**, **d** and **f**, closed and open circles stand for population growth in co-culture and monoculture experiments, respectively. In each co-culture experiment, strains were inoculated at 1:1 initial ratio. Experimental data are presented as mean (s.d.) from 3 independent experiments. In panels **g**, **h** and **i**, dashed lines correspond to monoculture growth, whereas solid lines correspond to co-culture growth.

analyzing their social interaction networks. For instance, in the AmA-AmB-CpAg consortium (Fig. 4c), AmA and CpAg both grew, but AmB went extinct because AmA and CpAg both produced nisin, which suppressed AmB; in contrast, in the AmA-AmB-CpBg consortium (Fig. 4d), all strains went extinct because AmA produced nisin, inhibiting both CpBg and AmB, and CpBg produced lcnA, suppressing AmA and AmB.

We further tested the feasibility of predicting complex community behaviors from the knowledge of two-strain consortia in selected four-strain ecosystems. Similarly to the three-strain cases, we extended the modeling framework to describe four four-strain communities formed through combinatorial strain mixing (Supplementary Note 3). Again, the only new parameters in the

models were the growth rates of the newly introduced strains. In parallel, we experimentally developed three new strains—PrBn, CmBn and AmBn—by removing the fluorescence reporter genes of strains PrB, CmB and AmB, respectively (Methods and Supplementary Table 1). Using the new and previous strains, we generated four four-strain ecosystems, performed their co-culture experiments, and further compared the measured population dynamics with the model predictions (Fig. 5a–d). These findings show that the models derived from the two-strain ecosystems successfully predicted the dynamics of more complex, four-strain ecosystems.

Although complex communities may involve higher order interactions, the agreement between the model predictions and

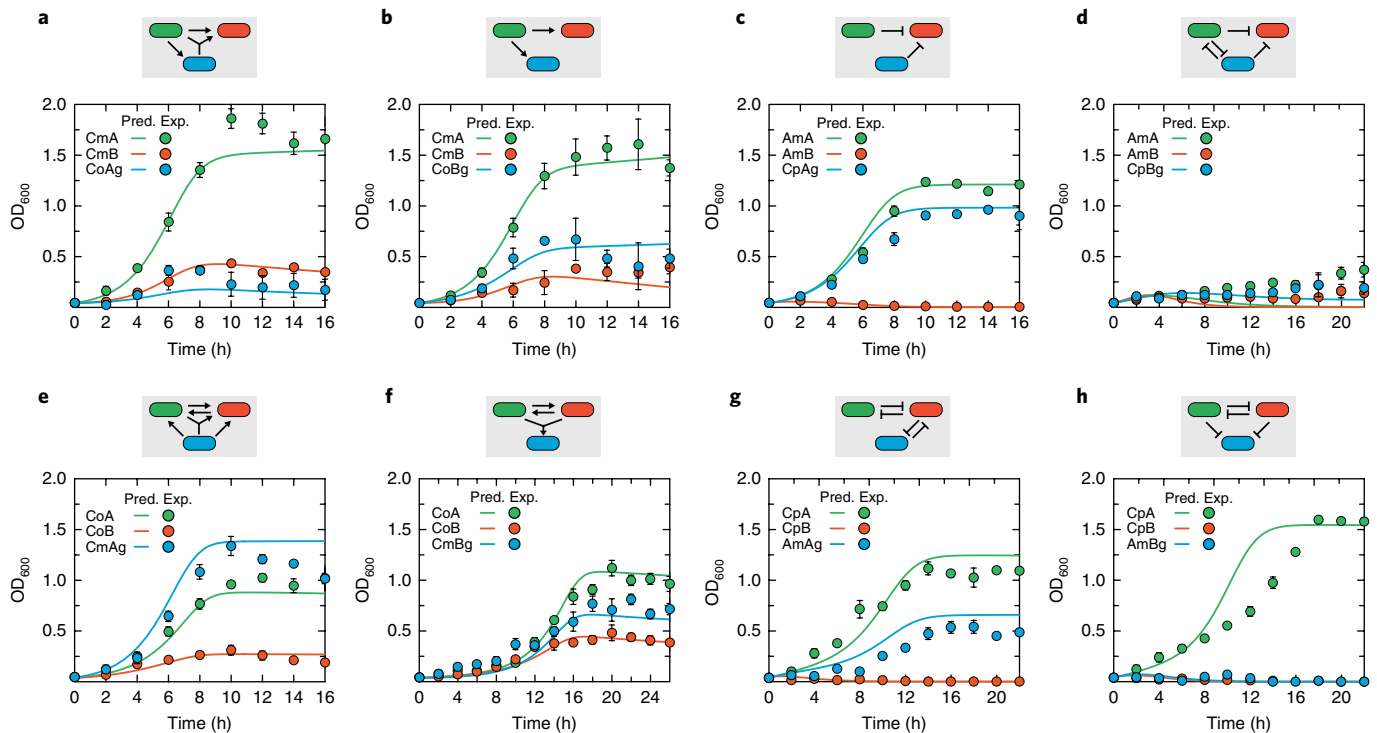


Fig. 4 | Model-predicted and experimentally measured population dynamics of three-strain ecosystems. **a**, Population dynamics of a three-strain consortium composed of the two commensalism strains (CmA and CmB) and a cooperation strain (CoAg). **b**, Population dynamics of a three-strain consortium composed of the two commensalism strains (CmA and CmB) and a cooperation strain (CoBg). **c**, Population dynamics of a three-strain consortium composed of the two amensalism strains (AmA and AmB) and a competition strain (CpAg). **d**, Population dynamics of a three-strain consortium composed of the two amensalism strains (AmA and AmB) and a competition strain (CpBg). **e**, Population dynamics of a three-strain consortium composed of the two cooperation strains (CoA and CoB) and a commensalism strain (CmAg). **f**, Population dynamics of a three-strain consortium composed of the two cooperation strains (CoA and CoB) and a commensalism strain (CmBg). **g**, Population dynamics of a three-strain consortium composed of the two competition strains (CpA and CpB) and an amensalism strain (AmAg). **h**, Population dynamics of a three-strain consortium composed of the two competition strains (CpA and CpB) and an amensalism strain (AmBg). Each ecosystem's interaction network is shown on the top of the corresponding panel. For all panels, lines and color circles stand for model predictions (pred.) and experimental (exp.) results, respectively. Data are presented as mean (s.d.) from 3 independent experiments. In all co-culture experiments, strains were inoculated at 1:1:1 initial ratio.

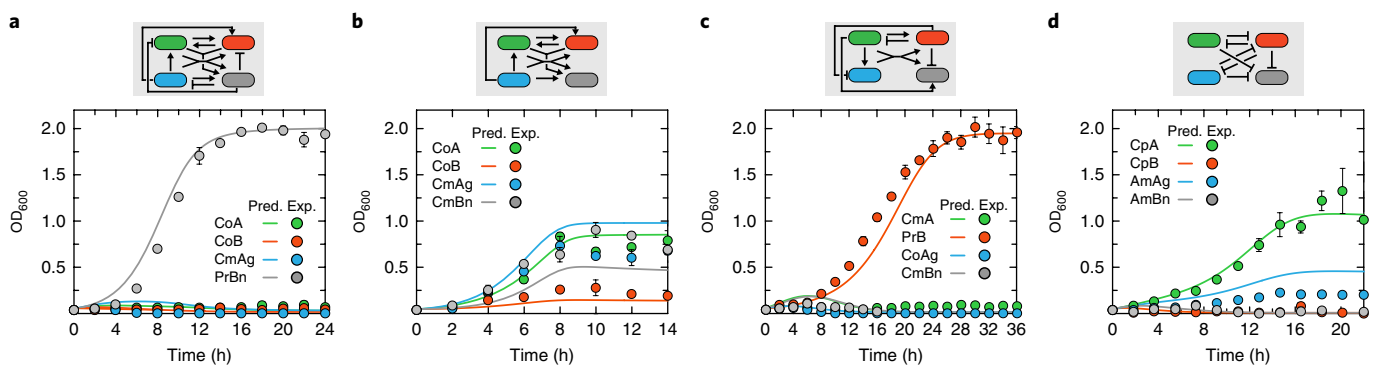


Fig. 5 | Model-predicted and experimentally measured population dynamics of four-strain ecosystems. **a**, Population dynamics of a four-strain consortium composed of two cooperation strains (CoA and CoB), a commensalism strain (CmAg) and a predation strain (PrBn). **b**, Population dynamics of a four-strain consortium composed of two cooperation strains (CoA and CoB) and two commensalism strains (CmAg and CmBn). **c**, Population dynamics of a four-strain consortium composed of two commensalism strains (CmA and CmBn), a predation strain (PrB) and a cooperation strain (CoAg). **d**, Population dynamics of a four-strain consortium composed of two competition strains (CpA and CpB) and two amensalism strains (AmAg and AmBn). Each ecosystem's interaction network is shown on the top of the corresponding panel. For all panels, lines and color circles stand for model predictions and experimental results, respectively. Data are presented as mean (s.d.) from 3 independent experiments. In all co-culture experiments, strains were inoculated at 1:1:1:1 initial ratio.

experimental measurements in Figs. 4 and 5 demonstrated that, at least for the ecosystems primarily containing pairwise interactions such as those tested, their community dynamics can be derived

from the behaviors of simpler consortia. Additionally, these results affirmed that social interaction engineering is an effective approach to program complex synthetic communities with desired dynamics.

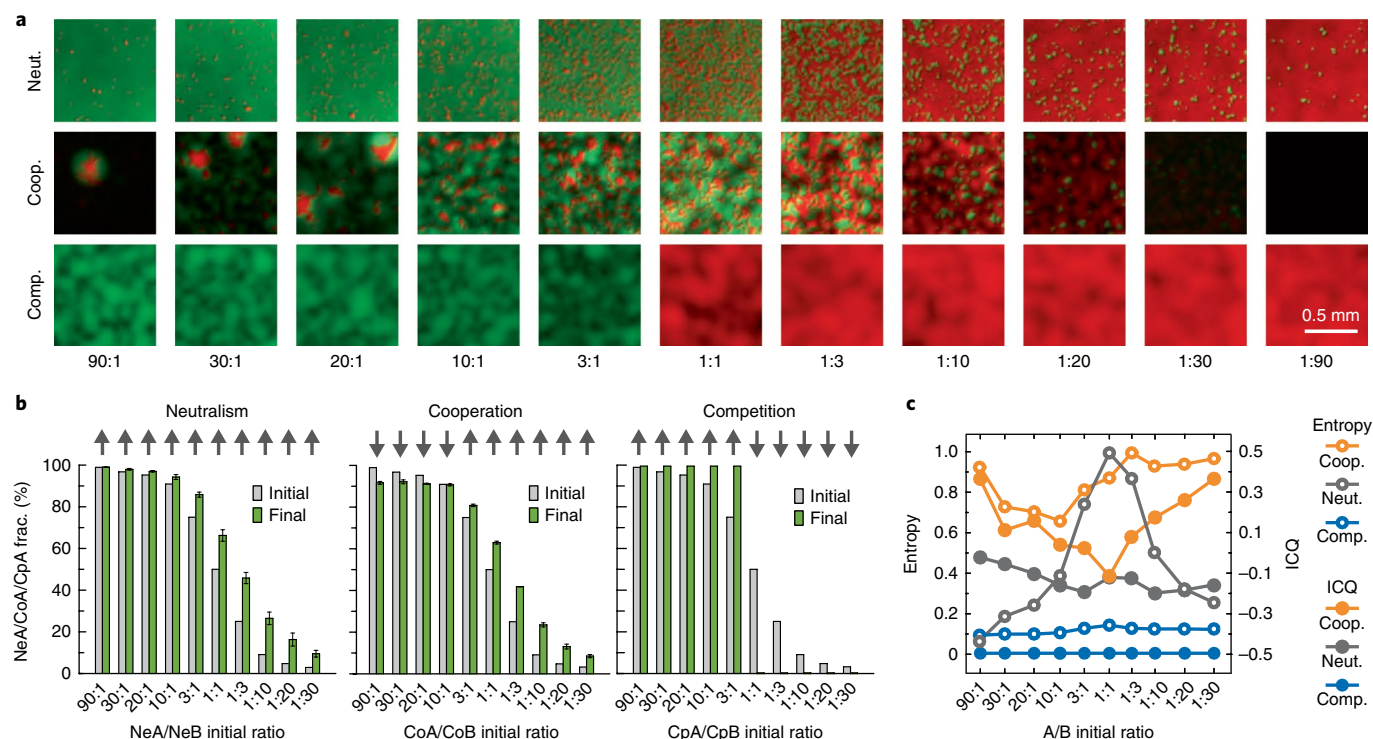


Fig. 6 | Spatial dynamics of three symmetrical communities. **a**, Spatial patterns emerged from the well-mixed consortia of neutralism, cooperation and competition growing on agar plates. A density of $OD_{600} = 0.2$ and varied ratios from 90:1 to 1:90 were used as initial conditions. Each experiment was repeated at least three times. Representative pictures from the experiments are shown. **b**, Comparison of the total initial and final population ratios of the consortia the experimental patterning processes. The NeA fraction increases in all cases due to a fast growth rate of NeA, the CoA fraction converged to ~90%, and CpA fraction diverged to either 100% or 0% depending on the initial conditions. Experimental data are presented as mean (s.d.) from 3 independent experiments. **c**, Entropy (open circles) and intensity correlation quotient (ICQ; closed circles) of the experimental patterns in panel **a**. The values of entropy and ICQ both follow the order of cooperation > neutralism > competition, although they are subject to initial conditions. Data are averaged from $n = 3$ experiments. Each experiment was repeated at least three times, and representative pictures from experiments are shown.

Spatial dynamics of three symmetrical ecosystems. In natural habitats, microbial communities such as the human gut microbiome and the rhizosphere microbiome often extend across a space wherein cellular interactions are subject to the diffusion of interacting molecules³⁷. This motivated us to examine whether social interactions play a similar role in spatial settings by using the consortia with symmetrical interactions—neutralism, cooperation and competition—as examples. Using a computational model involving reaction-diffusion equations (Methods; Supplementary Note 4 and Supplementary Table 7), we simulated the development of spatial structures of the three ecosystems whose initial cells were randomly seeded. Our results (Supplementary Figs. 8a and 9a) showed that strains (NeA and NeB) formed random patterns in neutralism, with their detailed cell distributions subject to initial seeding, relative abundance and growth rates; for the case of cooperation, the strains (CoA and CoB) tended to be colocalized in space and grew better with close initial ratios (3:1 to 1:3) than biased (90:1 to 10:1 and 1:10 to 1:90); in competition, homogeneous patterns of a single strain (CpA or CpB) emerged. Spatially averaged statistics of the initial and final populations in the three cases (Supplementary Fig. 8b) further suggested that the ecosystem population ratio drifts unidirectionally in neutralism, converges in cooperation and diverges in competition.

To test these model predictions, we performed a series of spatial patterning experiments using droplets of well-mixed consortia with varied strain ratios (90:1 to 1:90) but a fixed total density ($OD_{600} = 0.2$; Methods). Consistent with the modeling predictions, the strains within individual colonies were randomly distributed when neutral, colocalized in cooperation and mutually excluded in competition (Fig. 6a and Supplementary Fig. 9b). For comparison,

we also examined the spatiotemporal dynamics of the predation consortium, which showed an initial ratio-dependent pattern that is distinct from the symmetrical cases (Supplementary Fig. 10).

For the case of cooperation, spatial patterns were better developed with close initial ratios than with unbalanced ratios. However, we also noticed that CpB (red) in the competing consortium dominated in the spatial structures at the 1:1 ratio, contradicting the culture experiment in which CpA (green) won the contest (Fig. 3d). We speculated that the discrepancy arose from the different diffusibilities of nisin and lcnA, caused by the stronger hydrophobicity of the former, which was confirmed by patterning experiments on agar plates supplemented with Tween 20, a surfactant promoting nisin diffusion (Supplementary Fig. 11a). For the same reason, the initial ratio separators of the other three competing ecosystems all shifted toward a higher abundance of nisin producer (Supplementary Fig. 11b,c).

We further compared the relative abundances of the three symmetric consortia during pattern development. Similarly to the modeling analysis, the experimental results (Fig. 6b) showed that the NeA fraction of the neutral consortium increased over time for all initial conditions, the CoA fraction of the cooperative consortium converged toward 91% (i.e., 10:1 ratio) and the CpA fraction of the competitive consortium diverged (either 100% or 0%) with the transition occurring between 3:1 and 1:1. For comparison, population ratio changes in additional cases of competition were also calculated (Supplementary Fig. 11d–g). To quantify our findings, we further computed the entropy, a measure of the diversity of an ecosystem, and the intensity correlation quotient (ICQ), a measure of strain colocalization in space, of the experimentally observed (Fig. 6c) and simulated patterns (Supplementary Fig. 11h,i). These results

pointed to the existence of opposite population driving forces exerted by cooperation that promote coexistence and by competition that favor mutual exclusion.

In addition to well-mixed colonies, we investigated community organization in structured environments in which droplets of individual strains are spaced with varying distances (Supplementary Fig. 12a–c and Methods). For neutralism, we found that the strains (NeA and NeB) always coexisted. For cooperation, the strains (CoA and CoB) coexisted but their patterns decayed with the spacing from well-mixed filled circles (0 mm), to back-to-back domes (3 mm) and to diminishing edges (5 mm). For competition, emerged patterns shifted from one-strain exclusive circles (0 mm) to repelled two-strain domes (3 mm) and to full circles (5 mm) with the increase of droplet distance. For comparison, additional cases of competition were also experimentally tested (Supplementary Fig. 12d–g). The results confirmed that social interactions continue to serve as a driving force in structured settings; meanwhile, spatial factors, such as colony spacing in this case, function as additional regulators that modulate pattern emergence.

Discussion

With an increasing appreciation of microbiomes' profound impacts on human health, environment and agriculture, understanding and manipulating complex microbial ecosystems has become a defining mission for microbiome science. Our study provides fundamental insights into the structure, dynamics and ecology of interacting microbial species by characterizing synthetic consortia that serve as well-defined abstractions of native communities. In addition, our work demonstrates that social-interaction engineering, through the combination of modular pathway reconfiguration and model creation, is a systematic strategy to design ecosystem behaviors. Such a synthetic biology approach sets the stage for creating complex, community functions for a variety of applications. For example, toward microbial cell factories, social-interaction programming can be used to build the population stability of multiple synthetic strains co-involved in the division of labor in chemical synthesis by introducing cross-feeding or differentiating substrate utilizations; such augmented stability will boost the robustness and yield of chemical production during microbial fermentation. Social-interaction engineering also enables the establishment of synthetic ecosystems with predictable temporal and spatial dynamics, which can serve as a controllable tool to systematically perturb microbiomes and further alter their structure and dynamics in a desired manner. Looking forward, as microbes inhabit primarily complex, natural environments, our engineering strategy will become more versatile by validating the translatability of its applications from well-controlled, laboratory conditions to realistic settings.

Methods

Methods, including statements of data availability and any associated accession codes and references, are available at <https://doi.org/10.1038/s41589-018-0091-7>.

Received: 23 June 2017; Accepted: 10 May 2018;

Published online: 25 June 2018

References

- Brenner, K., You, L. & Arnold, F. H. Engineering microbial consortia: a new frontier in synthetic biology. *Trends Biotechnol.* **26**, 483–489 (2008).
- Großkopf, T. & Soyer, O. S. Synthetic microbial communities. *Curr. Opin. Microbiol.* **18**, 72–77 (2014).
- De Roy, K., Marzorati, M., Van den Abbeele, P., Van de Wiele, T. & Boon, N. Synthetic microbial ecosystems: an exciting tool to understand and apply microbial communities. *Environ. Microbiol.* **16**, 1472–1481 (2014).
- Bittihn, P., Din, M. O., Tsimring, L. S. & Hasty, J. Rational engineering of synthetic microbial systems: from single cells to consortia. *Curr. Opin. Microbiol.* **45**, 92–99 (2018).
- Weber, W., Daoud-El Baba, M. & Fussenegger, M. Synthetic ecosystems based on airborne inter- and intrakingdom communication. *Proc. Natl Acad. Sci. USA* **104**, 10435–10440 (2007).
- Balagaddé, F. K. et al. A synthetic *Escherichia coli* predator-prey ecosystem. *Mol. Syst. Biol.* **4**, 187 (2008).
- Chen, Y., Kim, J. K., Hirning, A. J., Josić, K. & Bennett, M. R. Emergent genetic oscillations in a synthetic microbial consortium. *Science* **349**, 986–989 (2015).
- Gore, J., Youk, H. & van Oudenaarden, A. Snowdrift game dynamics and facultative cheating in yeast. *Nature* **459**, 253–256 (2009).
- Chuang, J. S., Rivoire, O. & Leibler, S. Simpson's paradox in a synthetic microbial system. *Science* **323**, 272–275 (2009).
- Mee, M. T., Collins, J. J., Church, G. M. & Wang, H. H. Syntrophic exchange in synthetic microbial communities. *Proc. Natl Acad. Sci. USA* **111**, E2149–E2156 (2014).
- Wintermute, E. H. & Silver, P. A. Emergent cooperation in microbial metabolism. *Mol. Syst. Biol.* **6**, 407 (2010).
- Zhou, K., Qiao, K., Edgar, S. & Stephanopoulos, G. Distributing a metabolic pathway among a microbial consortium enhances production of natural products. *Nat. Biotechnol.* **33**, 377–383 (2015).
- Minty, J. J. et al. Design and characterization of synthetic fungal-bacterial consortia for direct production of isobutanol from cellulosic biomass. *Proc. Natl Acad. Sci. USA* **110**, 14592–14597 (2013).
- Hood, L. Tackling the microbiome. *Science* **336**, 1209 (2012).
- Cho, I. & Blaser, M. J. The human microbiome: at the interface of health and disease. *Nat. Rev. Genet.* **13**, 260–270 (2012).
- Falkowski, P. G., Fenchel, T. & Delong, E. F. The microbial engines that drive Earth's biogeochemical cycles. *Science* **320**, 1034–1039 (2008).
- Berendsen, R. L., Pieterse, C. M. & Bakker, P. A. The rhizosphere microbiome and plant health. *Trends Plant Sci.* **17**, 478–486 (2012).
- Faust, K. & Raes, J. Microbial interactions: from networks to models. *Nat. Rev. Microbiol.* **10**, 538–550 (2012).
- Shou, W., Ram, S. & Vilar, J. M. Synthetic cooperation in engineered yeast populations. *Proc. Natl Acad. Sci. USA* **104**, 1877–1882 (2007).
- Brenner, K., Karig, D. K., Weiss, R. & Arnold, F. H. Engineered bidirectional communication mediates a consensus in a microbial biofilm consortium. *Proc. Natl Acad. Sci. USA* **104**, 17300–17304 (2007).
- Hasty, J., McMillen, D. & Collins, J. J. Engineered gene circuits. *Nature* **420**, 224–230 (2002).
- Endy, D. Foundations for engineering biology. *Nature* **438**, 449–453 (2005).
- Andrianantoandro, E., Basu, S., Karig, D. K. & Weiss, R. Synthetic biology: new engineering rules for an emerging discipline. *Mol. Syst. Biol.* **2**, 2006.0028 (2006).
- Arkin, A. Setting the standard in synthetic biology. *Nat. Biotechnol.* **26**, 771–774 (2008).
- Brophy, J. A. & Voigt, C. A. Principles of genetic circuit design. *Nat. Methods* **11**, 508–520 (2014).
- Collins, J. J. et al. Synthetic biology: how best to build a cell. *Nature* **509**, 155–157 (2014).
- Purnick, P. E. & Weiss, R. The second wave of synthetic biology: from modules to systems. *Nat. Rev. Mol. Cell Biol.* **10**, 410–422 (2009).
- Hartwell, L. H., Hopfield, J. J., Leibler, S. & Murray, A. W. From molecular to modular cell biology. *Nature* **402**, C47–C52 (1999).
- Lubelski, J., Rink, R., Khusainov, R., Moll, G. N. & Kuipers, O. P. Biosynthesis, immunity, regulation, mode of action and engineering of the model lantibiotic nisin. *Cell. Mol. Life Sci.* **65**, 455–476 (2008).
- Stoddard, G. W., Petzel, J. P., van Belkum, M. J., Kok, J. & McKay, L. L. Molecular analyses of the lactococci A gene cluster from *Lactococcus lactis* subsp. *lactis* biovar diacetylactis WM4. *Appl. Environ. Microbiol.* **58**, 1952–1961 (1992).
- West, S. A., Diggle, S. P., Buckling, A., Gardner, A. & Griffin, A. S. The social lives of microbes. *Annu. Rev. Ecol. Syst.* **38**, 53–77 (2007).
- Foster, K. R. in *Social Behaviour: Genes, Ecology and Evolution* 331–356 (Cambridge Univ. Press, Cambridge, UK, 2010).
- Xavier, J. B. Social interaction in synthetic and natural microbial communities. *Mol. Syst. Biol.* **7**, 483 (2011).
- Kong, W., Kapuganti, V. S. & Lu, T. A gene network engineering platform for lactic acid bacteria. *Nucleic Acids Res.* **44**, e37 (2016).
- Kong, W. & Lu, T. Cloning and optimization of a nisin biosynthesis pathway for bacteriocin harvest. *ACS Synth. Biol.* **3**, 439–445 (2014).
- Volterra, V. Variations and fluctuations of the number of individuals in animal species living together. *ICES J. Mar. Sci.* **3**, 3–51 (1928).
- Nadell, C. D., Drescher, K. & Foster, K. R. Spatial structure, cooperation and competition in biofilms. *Nat. Rev. Microbiol.* **14**, 589–600 (2016).

Acknowledgements

We thank M. Sivaguru, G. Fried and A. Cyphersmith for their help with colony imaging at the IGB Core Facilities at UIUC, and B. Pilas of the Roy J. Carver Biotechnology

Center at UIUC for assistance with flow cytometry analysis in this study. We also thank H. Liu, W. van der Donk, X. Yang and A. Blanchard for stimulating discussions and help. This work was supported by the National Science Foundation (1553649, 1227034), the Office of Naval Research (N000141612525), the American Heart Association (12SDG12090025), the Center for Advanced Study at UIUC, National Center for Supercomputing Applications, the Paul G. Allen Frontiers Group, and the Defense Threat Reduction Agency (HDTRA1-14-1-0006).

Author contributions

T.L. and J.J.C. designed the study; T.L. conceived the project; W.K. performed the experiments and collected the data; D.R.M. developed the computational models; W.K., D.R.M. and T.L. analyzed the data; T.L., J.J.C., W.K. and D.R.M. discussed the results and wrote the paper.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information is available for this paper at <https://doi.org/10.1038/s41589-018-0091-7>.

Reprints and permissions information is available at www.nature.com/reprints.

Correspondence and requests for materials should be addressed to J.J.C. or T.L.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Methods

Strains. *Lactococcus lactis* NZ9000 was used as the host for strains in all ecosystems. Lactococcal strains were cultured in M17 medium with 0.5% glucose (GM17) at 30 °C. All plasmids were first constructed and sequenced in *E. coli* NEB-10 β , then transformed into *L. lactis* by electroporation. Antibiotics were added as required: chloramphenicol (10 $\mu\text{g ml}^{-1}$), erythromycin (250 $\mu\text{g ml}^{-1}$), tetracycline (3 $\mu\text{g ml}^{-1}$), kanamycin (50 $\mu\text{g ml}^{-1}$), spectinomycin (50 $\mu\text{g ml}^{-1}$), and streptomycin (150 $\mu\text{g ml}^{-1}$) for *E. coli*; chloramphenicol (5 $\mu\text{g ml}^{-1}$), erythromycin (5 $\mu\text{g ml}^{-1}$), and tetracycline (10 $\mu\text{g ml}^{-1}$) for *L. lactis*. Please see Supplementary Table 1 for full strain and plasmid information.

Construction of the reporter and selector plasmids. Oligos for plasmid construction are listed in Supplementary Table 2. All reporter and selector plasmids were developed from an *L. lactis*-*E. coli* shuttle vector, pleiss-Nuc, which contains a pSH71 origin, a chloramphenicol resistance gene, a PnisA promoter from a nisin gene cluster, and a Nuc reporter³⁸. Gibson assembly was used to construct all the reporter and selector plasmids. The plasmid for constitutively expressing GFP (pleiss-Pcon-gfp) was constructed by replacing PnisA promoter and Nuc in pleiss-Nuc with a *gfpuv* gene and the constitutive promoter of *lcnA*³⁰. The plasmid for constitutive expression of RFP, pleiss-Pcon-rfp, was constructed by replacing *gfp* in pleiss-Pcon-gfp with *mcherry*. The selector plasmids pleiss-Pnis-tet-Pcon-gfp and pleiss-Pnis-tet-Pcon-rfp were constructed by amplifying the *tet^R* gene from the plasmid pVPL3112 and Pcon-gfp or Pcon-rfp cassette from the plasmid constructed above and cloning them into pleiss-Nuc³⁹. The *tet^R* gene was under the control of the PnisA promoter. The plasmid pleiss-Pcon-tet-Pcon-gfp was constructed by insertion of *tet^R* and its RBS downstream of *gfp* in pleiss-Pcon-gfp. Both *gfp* and *tet^R* were under the control of the constitutive promoter of *lcnA*. The reporter and selector plasmids encoding a β -glucuronidase (*GusA*)⁴⁰ were constructed by replacing *gfp* in the plasmids pleiss-Pnis-tet-Pcon-gfp and pleiss-Pcon-tet-Pcon-gfp with *gusA*. The reporter-free selector plasmids were constructed by deleting the *gfp* gene from pleiss-Pnis-tet-Pcon-gfp and pleiss-Pcon-tet-Pcon-gfp.

Construction of the plasmids for nisin production and nisin resistance. All nisin-producing plasmids in ecosystems were constructed based on the plasmid pWK6, which was cloned by insertion of a wild-type nisin gene cluster from *L. lactis* k29 into pCCAM β 1; an *L. lactis*-*E. coli* shuttle vector^{34,35}. To avoid leaky expression of promoter PnisA in cells provided with multicopy *nisRK*, the *nisRK* gene in the multicopy plasmid pWK6 was knocked out. In brief, the plasmids pWK6 and the Red/ET recombination plasmid pRedET (GeneBridges) were transferred into NEB-10 β to generate the strain NEB-10 β /pWK6/pRedET. Then, the *aadA* gene (spectinomycin resistant gene) was amplified from plasmid pBeta³⁴, and the generating fragment was flanked with a short sequence of 3' of *nisP* and 5' of *nisF* and transformed into the induced competent cells of NEB-10 β /pWK6/pRedET using the protocol described previously³⁴. After recombination, the *nisRK* gene in pWK6 was replaced with the *aadA* gene, generating a new plasmid, pWK6-RK⁻. Then, pWK6-RK⁻ was transferred into *L. lactis* NZ9000 (a single copy *nisRK* in its chromosome) to test the complementation and recovery of the nisin-positive phenotype.

The plasmid pWK6 was also engineered to reduce its nisin productivity through reducing the RBS strength of *nisB* using ssDNA recombination performed as described previously³⁴. In brief, a 90-nt ssDNA oligo nisB269 embracing an RBS sequence with a translation initiation rate (TIR) of 269 AU was designed using RBS calculator (<https://salislab.net/software/>). The ssDNA was transferred to the Beta protein expressing NEB-10 β ::MutS/pBeta/pWK6 to replace the wild-type *nisB* RBS (TIR = 104,148 AU) through ssDNA recombination, thus generating the plasmid pWK6b. The plasmid pWK6b was further engineered to generate the *nisRK* knockout version pWK6b-RK⁻ according to the method described above. pWK6b-RK⁻ was also then transferred to *L. lactis* NZ9000 to test *nisRK* complementation.

The nisin-resistant plasmid was developed based on pWK6. First, the start codon and RBS of *nisA* were mutated in pWK6 by ssDNA recombination using a 90-nt oligo nisAmut. Second, *nisP* and *nisRK* were knocked out by selection and counterselection using a knstrepcassette, which contains kanamycin-resistant and streptomycin-sensitive genes, as described previously³⁴. Briefly, knstrepcassette flanking with 5' of *nisP* and 3' of *nisRK* was PCR amplified and transferred to NEB-10 β /pWK6/pRedET replacing *nisPRK*. A fragment generated by fusion of a short fragment of 5' of *nisP* and 3' of *nisRK* using overlap extension PCR (OE-PCR) was transformed into the strain to replace knstrepcassette by counter selection. Third, nisBTC was knocked out using the same method. The resulting plasmid was named pWK6-IFEG. Though pWK6-IFEG was unable to produce and modify nisin, it has all promoters and nisin immunity genes; it was transferred to *L. lactis* NZ9000 to test nisin immunity.

Construction of the plasmids for nisP⁻ nisin precursor producer and nisP⁺ nisin resistant strain. The NisP-deficient prenisin synthesis plasmid for cooperation was constructed from the plasmid pWK6b. First, *nisP* and *nisRK* genes in pWK6b were knocked out by replacing them with knstrepcassette. Second, a fragment combining 5' of *nisP* and 3' of *nisRK* was generated by overlap extension PCR and used to replace the knstrepcassette by counter selection. The

resulting plasmid was named pWK6b-PRK⁻. The NisP⁺ nisin-resistant plasmid for cooperation was generated from pWK6. First, the start codon and RBS of *nisA* were mutated in pWK6 by ssDNA recombination using the 90-nt oligo nisAmut. Second, *nisRK* were knocked out by selection and counterselection using the knstrepcassette. Third, nisBTC was knocked out by selection and counterselection. The resulting plasmid was named pWK6-IPFEG. Then, it was transferred to *L. lactis* NZ9000 to test nisin immunity. In addition, NZ9000/pWK6b-PRK⁻ and NZ9000/pWK6-IPFEG were co-cultured at a 1:1 ratio to examine their abilities in cooperative production of nisin.

Construction of the plasmid for lcnA producer. Genes of the *lcnA* gene cluster were amplified from plasmid pFI2396 and pFI2148 and assembled with P32 promoter into pleiss-Nuc vector in the following order: pleiss-3'-lcnA-lcnA-lcnA-promoter-5'-5'-P32-lcnA-3'-pleiss (P32-lcnA has a different direction with other genes in the cluster)⁴¹. The RBS TIR of the precursor gene *lcnA* was changed by designing an RBS sequence with different TIR using RBS calculator. Then, the *lcnA* gene cluster was amplified and subcloned into the *Not I* site of pCCAM β 1 by Gibson assembly. Two variants, pWK-lcnA^{5k} and pWK-lcnA^{20k}, with TIR of 5,078 AU and 19,950 AU, respectively, were chosen for subsequent experiments. In addition, genes of the *lcnA* gene cluster were also assembled to the wild-type gene cluster with their native promoters in the original order in pleiss-Nuc vector. The wild-type gene cluster was then transferred to pCCAM β 1 to generate the plasmid pWK-lcnA^{wt}.

Construction of the plasmid with nisin resistance and lcnA gene cluster.

The plasmid with both nisin resistance and *lcnA* production in predation was constructed by combining pWK-lcnA^{wt} and pWK6. First, pWK6 was engineered to create a *nisA* mutation (mutation of the start codon and RBS of *nisA*), *nisBTC* knockout and *nisPRK* knockout by ssDNA recombination and selection and counterselection as mentioned above. Then, the modified nisin gene cluster with only nisin resistance function was amplified and cloned into the *Not I* site of pWK-lcnA^{wt} through Gibson assembly. The resulting plasmid was named pWK6-IFEG-lcnA^{wt}.

Agar diffusion assay. All the nisin producer plasmids were transformed into *L. lactis* NZ9000. Agar diffusion assays were performed to measure nisin productivity of modified nisin producers and cooperative strains in cooperation, as well as *lcnA* productivity of *lcnA* producers. Agar diffusion assay was performed as previously described³⁴ except a new *L. lactis* 117 indicator strain was used. The plasmid pCCAM β 1 (Erm^R) and pleiss-Pcon-tet-Pcon-gfp (Cm^R Tet^R) were co-transformed to *L. lactis* 117 so that it was resistant to erythromycin, chloramphenicol and tetracycline simultaneously. The inhibition zone was then able to affect the concentration of nisin in the culture without interference of antibiotics.

Co-culture experiments. Bacterial strains were grown overnight in GM17 media containing chloramphenicol and erythromycin to an early stationary phase. Bacteria were centrifuged and washed twice with fresh GM17 media. Then, OD₆₀₀ of the cells was adjusted to 2.0. Each strain in co-culture or monoculture was inoculated to fresh media with appropriate antibiotics at a 1:50 dilution. For neutralism, amensalism and competition, erythromycin and chloramphenicol were added. For commensalism, cooperation and predation, erythromycin and tetracycline were added for selection. Samples were taken from the cultures every 2 h for measurement of growth. Meanwhile, cells from 500 μl of cultures were centrifuged and resuspended in PBS buffer. The cells were diluted to 10⁶ cells ml⁻¹ and stored in PBS buffer at 4 °C for at least 4 h and then vigorously vortexed, breaking most chains of lactococci into single cells. Then green and red fluorescent cells in the sample were counted by a Flow cytometer (BD LSR Fortessa). GFP was measured on the FITC channel, excited with a 488-nm blue laser and detected with a 530/30-nm bandpass filter. RFP was measured on PE-Texas Red channel using a 561-nm yellow/green laser and a 610/20-nm bandpass filter. At least 10,000 events were recorded for green and red cell counting per sample. In addition, images with green and red fluorescence were also taken by a fluorescence microscope and at least 1,000 cells from each sample were counted manually. The *GusA*-containing strains in the three- and four-strain consortia were distinguished by their blue color on GM17 agar plates containing 50 $\mu\text{g ml}^{-1}$ of X-gluc (5-bromo-4-chloro-3-indolyl β -D-glucuronide sodium salt), and their colony forming units (CFUs) were counted to calculate their ratio in the population. For the no-color strains in four-strain consortia, their populations were obtained by subtracting the numbers of green, red and blue cells from the total populations. The growth curve of each strain in the population was drawn by multiplying total OD₆₀₀ of the population with individual ratios. Control experiments with a single strain in commensalism, cooperation and predation induced with nisin were performed as follows: overnight cultures (diluted to OD₆₀₀ = 2.0) were inoculated to fresh GM17 media with erythromycin, tetracycline and 25 ng ml⁻¹ of nisin (1 IU = 25 ng) at a ratio of 1:50. The culture was then incubated at 30 °C, and cell densities were measured at 1–2 h intervals.

Spatial patterns of well-mixed consortium droplets. Overnight single-strain cultures for neutralism, cooperation and competition were centrifuged and washed

with fresh GM17 media. The cells were diluted to an OD_{600} of 0.2 using fresh media. Strain A and strain B in each ecosystem were mixed at different ratios from 90:1 to 1:90. Then, 1 μ l of the mixture was added onto a 90-mm agar plate (20 ml of GM17 agar with appropriate antibiotics and 2% agar). The plates were incubated at 30 °C for different times. The incubation time was determined by the growth rate of strains in neutralism, cooperation (induced with 25 ng/ml of nisin) and competition. The ratio of average growth rate of two strains in each consortium is 0.6 (cooperation): 0.79 (competition): 1 (neutralism) in liquid culture. Cooperation with the lowest growth rate formed clear laws on agar after 45 h growth. Then, the end point of the experiment of the other two ecosystems was calculated based on this time: 35 h (~0.76 of cooperation) for competition and 25 h (~0.6 of cooperation) for neutralism. Acquisition of images was performed with a Zeiss Axio V16 microscope using a HRm camera (Zoom 7 \times). The colonies grown from the droplets were subsequently picked up with an inoculating loop and dissolved in the PBS buffer. Ratios of strain A and strain B were counted by both plate pouring and flow cytometry. The spatiotemporal patterns of the predation ecosystem were obtained by spotting the well-mixed consortium droplets on the agar and taking images every 12 h with a Zeiss Axio V16 microscope.

Spatial patterns of structured consortium droplets. Overnight cultures were centrifuged and washed once with fresh media. The concentration of cells was diluted to an OD_{600} of 0.5 using fresh media. Then, 0.5 μ l of strain A and strain B in an ecosystem (neutralism, cooperation and competition) was added to a 90-mm agar plate (20 ml of 2% GM17 agar with appropriate antibiotics) with different distances. The plates were incubated at 30 °C for different times (25 h for neutralism, 45 h for cooperation and 35 h for competition). Acquisition of images was performed with a Zeiss Axio V16 microscope using a HRm camera (Zoom 7 \times).

Culture simulations. A general framework was constructed to describe the population dynamics of any two-strain community in which the strains consume a common nutrient and interact through signaling molecules. Well-mixed culture models for six different social interactions (commensalism, amensalism, neutralism, cooperation, competition and predation) were first constructed modularly from the framework. These models were fit to experimental data, then recombined modularly to predict three- and four-strain ecosystems. Whenever possible equations and parameters were reused to reflect the modular construction of the ecosystems. MATLAB was used to produce plots, analyze images and fit data for the models. Data from supplemental control experiments were also used to fit parameters.

Spatial simulations. A general framework was constructed to describe the spatiotemporal dynamics of any two-strain community that involves nutrient

co-utilization and active social interactions through diffusible signaling molecules. All three symmetric ecosystems (neutralism, cooperation and competition) were constructed modularly to reflect the modular reconfiguration concept of the ecosystem engineering. Each ecosystem was simulated using C++ and analyzed in MATLAB. The entropy and colocalization of bacterial strains were also measured and plotted.

Statistical analysis. All of the experiments were performed for at least three times. Replicate numbers of the experiments (n) are indicated in the figure legends. Sample sizes were chosen based on standard experimental requirement in molecular biology. Data are presented as mean \pm s.d. Fluorescence microscopy images are representatives of the images from multiple experimental replicates.

Reporting Summary. Further information on experimental design is available in the Nature Research Reporting Summary linked to this article.

Code availability. Custom C++ and MATLAB codes developed in this study are available within the paper and its supplementary information files or from the corresponding author upon reasonable request.

Data availability. Data supporting the findings of this study are available within the paper and its supplementary information files or from the corresponding author upon reasonable request. Sequences of plasmids are available at GenBank under the following accession numbers: pWK6, [MG913135](#); pWK6-RK, [MG913136](#); pWK6b-PRK, [MG913137](#); pWK-lcnA^{wt}, [MG913138](#); pWK-lcnA^{sk}, [MG913139](#); pWK-lcnA^{20k}, [MG913140](#); pWK6-IFEG, [MG913141](#); pWK6-IPFEG, [MG913142](#); pWK6-IFEG-lcnA^{wt}, [MG913143](#).

References

38. Le Loir, Y., Gruss, A., Ehrlich, S. D. & Langella, P. A nine-residue synthetic propeptide enhances secretion efficiency of heterologous proteins in *Lactococcus lactis*. *J. Bacteriol.* **180**, 1895–1903 (1998).
39. Oh, J.-H. & van Pijkeren, J.-P. CRISPR-Cas9-assisted recombineering in *Lactobacillus reuteri*. *Nucleic Acids Res.* **42**, e131 (2014).
40. Douglas, G. L. & Klaenhammer, T. R. Directed chromosomal integration and expression of the reporter gene *gusA3* in *Lactobacillus acidophilus* NCFM. *Appl. Environ. Microbiol.* **77**, 7365–7371 (2011).
41. Fernández, A., Horn, N., Gasson, M. J., Dodd, H. M. & Rodríguez, J. M. High-level coproduction of the bacteriocins nisin A and lactococcin A by *Lactococcus lactis*. *J. Dairy Res.* **71**, 216–221 (2004).

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see [Reporting Life Sciences Research](#). For further information on Nature Research policies, including our [data availability policy](#), see [Authors & Referees](#) and the [Editorial Policy Checklist](#).

► Experimental design

1. Sample size

Describe how sample size was determined.

Each experiment was performed at least 3 times. The number of biological replicates performed was chosen based on standard bacterial experiments reported in literature.

2. Data exclusions

Describe any data exclusions.

No data was excluded from analysis.

3. Replication

Describe whether the experimental findings were reliably reproduced.

Attempts on reproducing the results were successful.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

Not applicable.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

Researchers were not blinded during experiments because blinding is not applicable to this study.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a Confirmed

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
- A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- A statement indicating how many times each experiment was replicated
- The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
- A description of any assumptions or corrections, such as an adjustment for multiple comparisons
- The test results (e.g. P values) given as exact values whenever possible and with confidence intervals noted
- A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
- Clearly defined error bars

See the web collection on [statistics for biologists](#) for further resources and guidance.

► Software

Policy information about [availability of computer code](#)

7. Software

Describe the software used to analyze the data in this study.

Data fitting and analysis was done with custom matlab scripts. Additional modeling was done in C with matlab used to analyze simulation results.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* [guidance for providing algorithms and software for publication](#) provides further information on this topic.

► Materials and reagents

Policy information about [availability of materials](#)

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

All materials are commercially available. The strains or plasmids constructed in this study are available upon request from the author.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

No antibodies were used.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

No eukaryotic cells.

b. Describe the method of cell line authentication used.

No eukaryotic cells.

c. Report whether the cell lines were tested for mycoplasma contamination.

No eukaryotic cells.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by [ICLAC](#), provide a scientific rationale for their use.

No eukaryotic cells.

► Animals and human research participants

Policy information about [studies involving animals](#); when reporting animal research, follow the [ARRIVE guidelines](#)

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

No animals.

Policy information about [studies involving human research participants](#)

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

No human research participants.