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pleiotropic effects in progeny. Cleverly designed sensors like these may prove to be useful in studies of cell differentiation and decision-making, where cells are thought to progress through a continuum of poorly understood cellular states.

Controlling multicellular development and genetic inheritance. Recent work in *Drosophila* has shown that synthetic circuits can fundamentally alter the development and life cycle of a multicellular organism in a controlled way. Chen *et al.* created a synthetic selfish genetic element, named *Medea*, capable of spreading through a population (38). The synthetic *Medea* element (Fig. 4A) maternally expresses a microRNA (miRNA) that blocks expression of an essential protein normally produced by the mother and deposited in the egg. The element also expresses an “antidote” to this toxic miRNA, which consists of a second copy of the gene (with different codons) expressed by the embryo rather than the mother. Replacing the maternally expressed gene with its zygotically expressed *Medea*-based counterpart maintained normal development in offspring. *Medea*-positive mothers always express the toxic maternal miRNA. Thus, progeny of such mothers only survive if they inherit *Medea* from either or both parents—a dramatically non-Mendelian inheritance pattern.

A key consequence is that *Medea* is capable of invading populations. When *Medea*-positive flies are introduced into a wild-type laboratory population, the *Medea* element rapidly takes over the whole population (38). A similar synthetic system in mosquitos could in principle be engineered to carry a “cargo” gene that would diminish the ability of malarial parasites to survive in the mosquito or to be transmitted to human hosts (Fig. 4C).

A striking aspect of the *Medea* system is that it works across multiple levels: At the circuit level, it rewires expression of a critical gene to alter the timing and genetic source of expression (Fig. 4A). At the developmental level, this leads to a selective

killing of embryos that lack the *Medea* element (Fig. 4B). Finally, at the population level, this gives *Medea* transgenic organisms the ability to efficiently spread through a population (Fig. 4C). Although many challenges remain, this system and others [see (39, 40)] demonstrate the power of integrating synthetic biology approaches into the circuitry of a complex organism.

Conclusion: Exploring the Biology That Could Be

Synthetic biology opens up the possibility of creating circuits that would not survive in the natural world and studying their behaviors in living cells, expanding our notion of biology (41). The last decade has shown how even our first steps toward building and analyzing synthetic circuits can identify fundamental biological design principles and can produce useful new understanding. Future progress will require work across a range of synthetic levels (Fig. 1), from rewiring to building autonomous and integrated circuits de novo. Going forward, we anticipate that synthetic biology will become one of the primary tools we use to understand, control, imagine, and create biological systems.

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REVIEW

Synthetic Biology Moving into the Clinic

Warren C. Ruder,* Ting Lu,* James J. Collins†

Synthetic biology is an emerging field focused on engineering biomolecular systems and cellular capabilities for a variety of applications. Substantial progress began a little over a decade ago with the creation of synthetic gene networks inspired by electrical engineering. Since then, the field has designed and built increasingly complex circuits and constructs and begun to use these systems in a variety of settings, including the clinic. These efforts include the development of synthetic biology therapies for the treatment of infectious diseases and cancer, as well as approaches in vaccine development, microbiome engineering, cell therapy, and regenerative medicine. Here, we highlight advances in the biomedical application of synthetic biology and discuss the field’s clinical potential.

A little over a decade ago, the development of two engineered gene networks—a toggle switch (1) and an oscillator (2)—set in motion the rapid emergence of synthetic biology

as a field. In the years following, increasingly sophisticated synthetic gene circuits have been designed and constructed. Inspired by electrical circuits as well as natural biomolecular networks, these

devices include timers, counters, clocks, logic processors, pattern detectors, and intercellular communication modules (3–9). These DNA-encoded synthetic circuits are typically uploaded into cells, with their programmable abilities allowing for the precise control of cellular behavior and phenotype.

Meanwhile, there is a growing need for the development of new, important medical treatments. Bacteria, for example, are becoming resistant to antibiotics faster than we can develop effective replacements (10). Additionally, surgery remains a common cancer treatment, and when

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radiation and chemotherapy do work, patients suffer off-target effects. Customized therapies that can be designed to interact with a patient's physiology in prescribed ways are needed.

The field of synthetic biology is beginning to use its methods and platforms to bring engineering approaches into biomedicine. Effective synthetic biology therapies are being rationally designed and implemented as researchers build constructs (e.g., engineered biomolecules, synthetic gene networks, and programmable organisms) to alter mechanisms underlying disease and related biological processes (Fig. 1). Here, we highlight synthetic biology strategies that have been developed to target infectious diseases and cancer, as well as approaches in vaccine development, microbiome engineering, cell therapy, and regenerative medicine. We conclude by discussing how future work in synthetic biology could affect biomedicine and by describing the challenges that need to be overcome for the field to translate its promise into practice.

Treatment and Prevention of Infections

In addressing the need to develop strategies to enhance our antimicrobial arsenal, synthetic biology constructs have been developed to treat bacterial infections, as well as improve the efficacy of existing antibiotics. For example, bacteriophage—viruses that only infect specific bacteria—have been engineered to attack or weaken resistant bacterial strains by disrupting antibiotic defense mechanisms.

In an initial study, enzymatic bacteriophage were engineered to degrade bacterial biofilms and kill off bacterial cells in the biofilm (11). Biofilms, which play a critical role in the pathogenesis of many persistent infections, are surface-associated bacterial communities encapsulated in an extracellular polymeric matrix that shields bacteria from attack by host immune defenses and antibiotics. Lytic T7 phage were engineered to express the biofilm-matrix-degrading enzyme dispersin B (DspB) as well as rapidly replicate during infection. In a two-pronged attack, bacterial lysis induced by the engineered phage killed the infected bacterial cells in the biofilm and dispersed DspB along with the newly produced phage. The released DspB degraded the biofilm matrix, which exposed newly unprotected bacteria to the released phage, resulting in a cyclic process that eventually removed 99.997% of bacterial cells in treated biofilms.

In a second study, synthetic adjuvants were designed by engineering bacteriophage to enhance the killing efficacy of existing antibiotics (12). This approach focused on disrupting bacterial networks that regulate antibiotic defense mechanisms. All bactericidal antibiotics induce DNA damage, resulting in the activation of the SOS response network (13). Nonlytic M13 phage, chosen to minimize activation of bacterial adaptation mechanisms, were engineered to inhibit the damage response by overexpressing *lexA3*,

a repressor of the SOS network (Fig. 2A). Phage treatment resulted in significantly enhanced killing of bacterial strains by three major classes of antibiotics, that is, quinolones, β -lactams, and aminoglycosides. For example, in vitro treatment with engineered phage and the quinolone ofloxacin resulted in a 5000-fold increase in the killing of resistant bacteria compared to treatment with the antibiotic alone. In an animal study, treatment with engineered phage and ofloxacin resulted in an 80% survival rate in *Escherichia coli*-infected mice, compared to 20% with antibiotic treatment alone.

Synthetic constructs can also be designed to limit the spread of infection by targeting disease vectors. Along these lines, Crisanti and colleagues recently attempted to reduce malaria transmission by rationally modifying the disease's mosquito vector using a synthetic biology approach. Specifically, they built a synthetic construct that could, in principle, enable a laboratory mosquito population to rapidly disseminate a genetic modification (e.g., disruption of genes encoding malaria vector capability) to a field population (14).

This transgenically introduced construct—a synthetic, homing endonuclease-based gene (HEG) drive—consisted of mosquito regulatory regions and a homing endonuclease gene, *I-SceI* (fig. S1). The gene drive first used endonuclease to induce double-strand DNA breaks that activated the recombinational DNA repair system in mosquito cells. The homologous chromosome, carrying the HEG (and potentially any other synthetic or endogenous gene), was then used as a template for repair, resulting in both chromosomes carrying the synthetic drive. The HEG drive rapidly spread in transgenic cage populations that carried corresponding endonuclease recognition sites, matching analytical model predictions, and molecular analyses showed high rates of chromosomal cleavage and conversion. For the eventual deployment of this system in the wild, the synthetic HEG drive

will require, among other things, identifying or engineering a homing endonuclease with recognition sites in the native vector genome. Of note, homing endonucleases have been designed to target specific DNA sequences for potential genome engineering and gene therapy (15, 16). Alternatively, in addition to targeted disruptions, new genes could be distributed to suppress malaria vector capacity. In a review, Nandagopal and Elowitz (17) describe a synthetic *Medea* system inspired by natural gene drives (18), which quickly distributed genetic cargo to wild *Drosophila* populations.

Cancer Treatment

Despite the success of modern cancer therapies, the three major therapeutic interventions—surgery, radiation, and chemotherapy—still typically result in considerable damage to healthy tissue. We need new cancer treatments that precisely distinguish between diseased and healthy cells. To this end, synthetic biologists have engineered bacteria to target and invade cancer cells. In one study, the invasion was designed to occur only in specific tumor-related environments, whereas in another, the bacterial invaders were engineered to knock down a specific, endogenous cancer-related gene network.

In the first study, Voigt and colleagues engineered bacteria to invade cancer cells only in the hypoxic environment often indicative of tumor tissue (19). Cell-invasion ability was enabled in *E. coli* by engineering them to express the invasin (*inv*) adhesion protein from *Yersinia pseudotuberculosis*, which tightly binds mammalian $\beta 1$ integrin receptors, inducing uptake. Invasin expression was placed under the control of an anaerobically induced formate dehydrogenase promoter, resulting in bacteria that only invaded mammalian cell cultures in hypoxic environments. Tissue is typically hypoxic, however, when it has no access to blood, which could limit the efficacy of intravenously delivered,

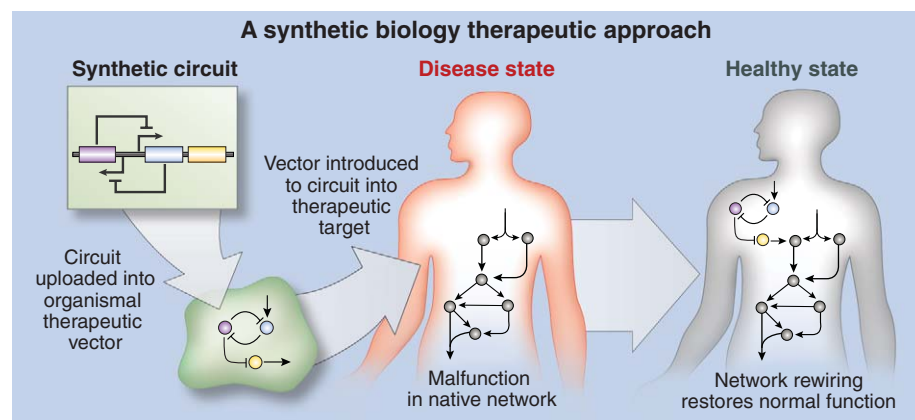


Fig. 1. Synthetic circuit development for the treatment of disease. Synthetic gene networks are uploaded into cells to therapeutically target the body's endogenous networks, causing a transition from disease to healthy state. Here, the uploaded network is a bistable toggle switch, which enables cellular memory with a network of two mutually repressible modules.

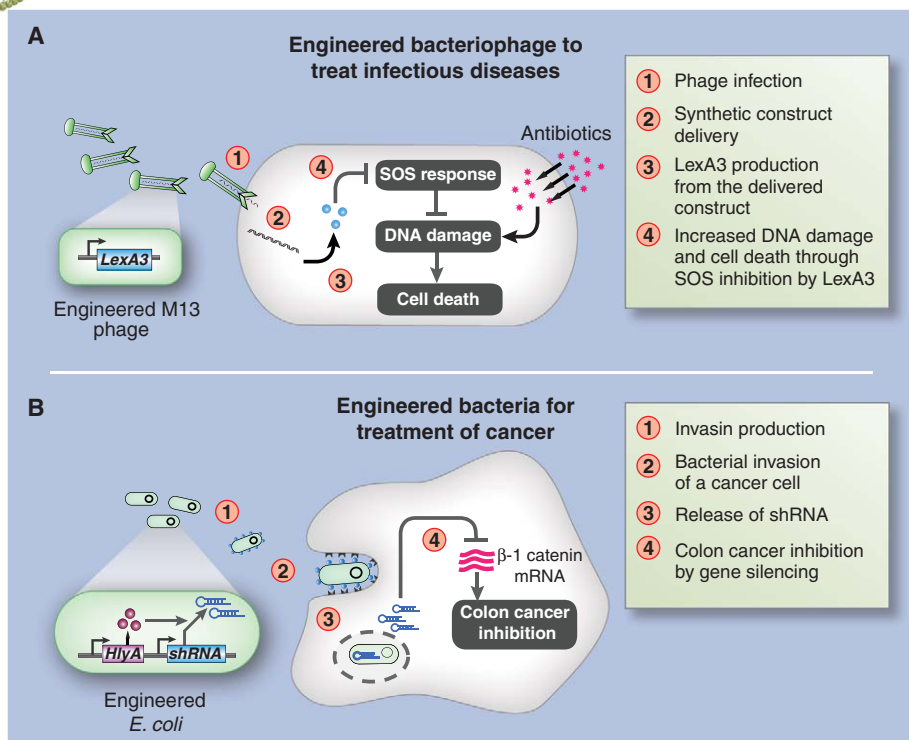


Fig. 2. Synthetic biology approaches for treatment of bacterial infection and cancer. **(A)** Engineered bacteriophage boosted antibiotic killing efficacy by disrupting repair of antibiotic-induced damage. **(B)** Engineered bacteria invaded cancer cells and knocked down a cancer gene using RNAi.

cancer-targeting bacteria. Also, given the dynamics of blood flow, the bacteria would need to be engineered to quickly express invasin and enter cells.

In the second study, Li and colleagues were able to intravenously deliver engineered, cancer-invading bacteria to target a specific tumorigenic pathway in vivo (20). Using RNA interference (RNAi), bacterial invaders were designed to knock down expression of *CTNNB1* (encoding β -1 catenin), a gene that initiates many colon cancers upon its overexpression or oncogenic mutation (Fig. 2B). The engineered bacteria accomplished gene knockdown by generating short hairpin RNA (shRNA) segments that bound to *CTNNB1* mRNA transcripts and induced mRNA cleavage. Along with the shRNA and invasin, the synthetic system produced lysteriolysin O (encoded by the *hlyA* gene), which enables molecular transport out of vesicles in a process believed to involve entry vesicle disruption.

Bacteria cells uploaded with the synthetic circuitry robustly invaded colon cancer cells in vitro and knocked down *CTNNB1* expression. Intravenous administration of the engineered *E. coli* into immune-deficient mice with subcutaneously xenografted human colon cancer cells resulted in significant knockdown of the gene in tumor cells, showing that bacterial invaders could be directed to distal cancer targets. In the future, the two synthetic constructs described above could be coupled, potentially producing programmable

bacteria that invade cancer cells under specific in vivo conditions and, once inside, target specific cancer-related pathways.

Vaccine Development

The development of new vaccines is limited by several drawbacks, including risks associated with the use of attenuated pathogens, along with difficulties altering vaccine target specificity. To address these issues, Mastrobattista and co-workers used liposomes—synthetic vesicles consisting of a lipid bilayer—to encapsulate a combination of a reconstituted bacterial transcription-and-translation network and DNA encoding a model antigen (β -galactosidase) (21). The system (fig. S2) produced functional antigen protein in vitro. In live mice, antigen-expressing liposomes generated a higher humoral immune response compared with control vaccines (liposomes encapsulating only the antigen, the transcription-and-control network, or the DNA template, respectively). This system can be easily altered for other antigens by simply changing the DNA template and carries no risk of infection by attenuated pathogens.

Additional progress in the field may come from combining synthetic circuits with recent genomic engineering advances in vaccine development. For example, Wimmer and colleagues attenuated poliovirus by exploiting species-specific bias for codon pairs (22). Although DNA codons are synonymous (several different codons can encode a single amino acid), every species has a

bias for the adjacent codon pairs it can translate efficiently into protein.

To exploit this bias, hundreds of synonymous codon pairs were switched in the gene sequence encoding the poliovirus capsid protein, resulting in decreased translational efficiency. The resulting inefficient, attenuated virus was sufficient to provide protective immunity after challenge. However, in this case, the DNA encoding the capsid protein was altered through de novo synthesis and reinserted into living cells. If a synthetic circuit could be designed to automatically swap synonymous codons in the genome of infected cells, a completely cell-based system for virus attenuation would be possible.

Microbiome Engineering

The human microbiome—the microorganisms associated with the human body—is a complex ecosystem increasingly implicated as a regulator of host physiology. It numbers over 1000 species and outnumbers human cells by a factor of 10 to 100 (23). As microbiome constituents are typically well-tolerated, naturally commensal microorganisms, they are potentially excellent vectors for deploying synthetic gene circuits to fight disease and correct aberrant conditions. Social interactions within and between species also play a critical role in microbiome communities (24, 25) and could be harnessed.

Along these lines, Duan and March recently used *E. coli* to prevent cholera infection by engineering a synthetic interaction between gut microbes (26). During cholera infection, *Vibrio cholerae* secrete virulence factors, such as cholera toxin (CT), only at low population density. To assess its own density, *V. cholerae* uses quorum sensing, a process in which autoinducer signaling molecules are both secreted and detected by members of a population. *V. cholerae* detects levels of cholera autoinducer 1 (CAI-1) and autoinducer 2 (AI-2), and when both are high, ceases expression of virulence factors. Duan and March took advantage of this mechanism and engineered *E. coli* that produce AI-2 to also secrete CAI-1 (Fig. 3). When infant mice ingested the engineered *E. coli* 8 hours before *V. cholerae* ingestion, their survival rate increased dramatically and cholera toxin intestinal binding was reduced by 80%.

Alternatively, a patient’s microbiome could be engineered to deliver therapeutic molecules directly to the body. For example, commensal bacteria strains have been engineered to secrete key molecules for potential disease treatment, including insulinotropic proteins for diabetes (27), an HIV fusion inhibitor peptide for prevention of HIV infection (28), and interleukin-2 for immunotherapy (29). Although these studies showed effective expression of therapeutically relevant molecules, each would benefit from the development and use of synthetic circuits. By placing, for example, the expression of therapeutic molecules under the control of cell-based sensors that detect

aberrant, pathological conditions, gene expression could be turned on and tuned accordingly only when the prescribed molecular interventions are needed, reducing metabolic load on the bacteria and increasing their ability to assimilate into the microbiome.

Cell Therapy and Regenerative Medicine

Cell therapy—the introduction of prescribed cells into the body to treat disease—is promising, yet challenges remain due to an inability to control post-implantation cell behavior and phenotype. One solution could involve uploading synthetic circuits into cells before implantation, thus endowing them with sophisticated control systems. Unfortunately, the great majority of synthetic gene circuits designed thus far have been limited to microbes. The recent extension of synthetic circuits to mammalian cells, however, has opened the door to new and enhanced cell therapies.

Tight control of specific genes is critical for effective cell therapies. To address this problem, we recently developed a tunable, modular mammalian genetic switch (30). This entailed creating a synthetic gene network that couples repressor proteins with an RNAi design involving shRNA. Gene expression is turned on by adding an inducer, which controls the repressor elements at the transcriptional level, while simultaneously turning off the RNAi component to allow the transcript to be retained and translated (fig. S3). The switch offers >99% repression, as well as the ability to tune the expression of the gene of interest. Modular capabilities of the system allow for the regulation of any gene, as well as the potential for tissue-specific use (its genetic elements can be controlled by tissue-specific promoters). The switch was validated in mouse and human cells. This tight, tunable, and reversible control of mammalian gene expression could be used in cell therapy applications, as well as to determine whether a disease phenotype is the result of a threshold response to changes in gene expression.

Fussneger and colleagues recently designed a synthetic mammalian gene circuit to regulate uric acid homeostasis *in vivo*, the disturbance of which is associated with tumor lysis syndrome and gout (31). This synthetic device sensed uric acid using an engineered repressor that could be induced (i.e., derepressed) by uric acid. Upon derepression, the network expressed an engineered urate oxidase that eliminated uric acid (Fig. 4A). Circuit-expressing cells implanted in urate oxidase-deficient, transgenic mice decreased urate concentrations to subpathological levels and reduced uric acid crystal deposits in the kidneys.

Shifting from transcriptional control to translational control, Smolke and colleagues constructed a synthetic device using a drug-responsive-RNA module for gene regulation in mammalian cells (32). In mice, the RNA device controlled T cell proliferation by linking a drug-responsive ribozyme to growth cytokine expression. Program-

ming cells to execute sophisticated processes upon implantation could eventually allow synthetic gene circuits to be customized for individual patients.

The tailoring of engineered cells to a patient's physiology will also be critical in the field of regenerative medicine, where the eventual gold standard therapy likely will involve tissues created from a patient's own stem cells. Although the adult body maintains clinically accessible niches for some stem cell lineages (e.g., hematopoietic and adipogenic), many others are difficult to access. With the development of induced pluripotent stem cells (iPSCs), adult-derived stem cells that, in principle, could be differentiated into any cell type are now available. iPSCs can be created from an adult patient's cells upon the insertion and expression of only four genes [e.g., KLF4, c-MYC, OCT4, and SOX2 (KMOS)] (33), a breakthrough methodology that nonetheless comes with concerns and drawbacks (34). For example, virally introduced extra copies of all four genes must be inserted permanently into the cellular genome, which can make such cells prone to forming tumors.

Rossi and colleagues recently addressed this problem by adopting a synthetic biology approach and chemically transfecting cells with synthetic, modified RNA molecules that function as mRNA transcripts for the four key genes (35). Once inside cells, the transcripts are translated into proteins that induce pluripotency without the integration of extra genes into the genomes. Using this method, investigators were able to create iPSCs faster and with a greater yield than viral delivery (Fig. 4B). The team also used this method to create RNA-iPSCs (RiPSCs) from multiple human cell types and further showed that the same technology can efficiently direct RiPSCs to terminally differentiate into myogenic cells. In the future, it will be exciting to see whether synthetic biology approaches can create constructs

that enable targeting and reprogramming of injured, diseased, or aged tissue *in vivo*.

Outlook

Although synthetic biology is in its infancy as a field, its practitioners are taking initial steps toward developing new biomedical therapies. The field initially arose from the combined efforts and insights of a small band of engineers, physicists, and computer scientists whose backgrounds dictated the early directions of synthetic biology. For the field to reach its full clinical potential, it must become better integrated with clinicians.

Clinical applications will surely necessitate increasingly complex circuits and constructs. Up to this point, the field has developed circuits using, more or less, the same collection of basic regulatory components. However, in order to build more complicated, clinically applicable circuits, it will be necessary to identify entirely new modules and components from endogenous networks as well as to synthesize and characterize diverse component libraries. Additionally, although most synthetic systems have been transcriptional, post-transcriptional systems, particularly protein-based systems, will be needed to enable faster responses. Along these lines, Voigt and colleagues have engineered protein-based light sensors and used them to activate mammalian cell signaling (36). We also will need more effective computational tools to fast-track synthetic biology, both for identifying new components and predicting the behavior of complicated synthetic systems.

Moreover, there exists a critical need to move synthetic biology increasingly toward mammalian systems. Most synthetic constructs have been deployed in microbes; however, many clinical problems will require mammalian circuits, components, and constructs. An expanded mammalian toolbox would enable synthetic biology to address a broader range of applications in

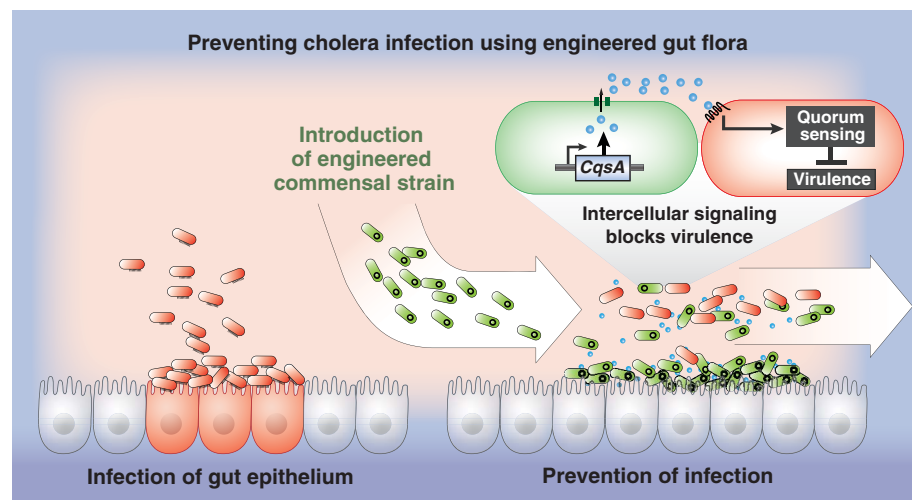


Fig. 3. Synthetic biology approach for microbiome engineering. Native commensal bacteria were engineered to secrete the molecular signal cholera autoinducer (CAI-1), which leads to inhibition of *V. cholerae* virulence.

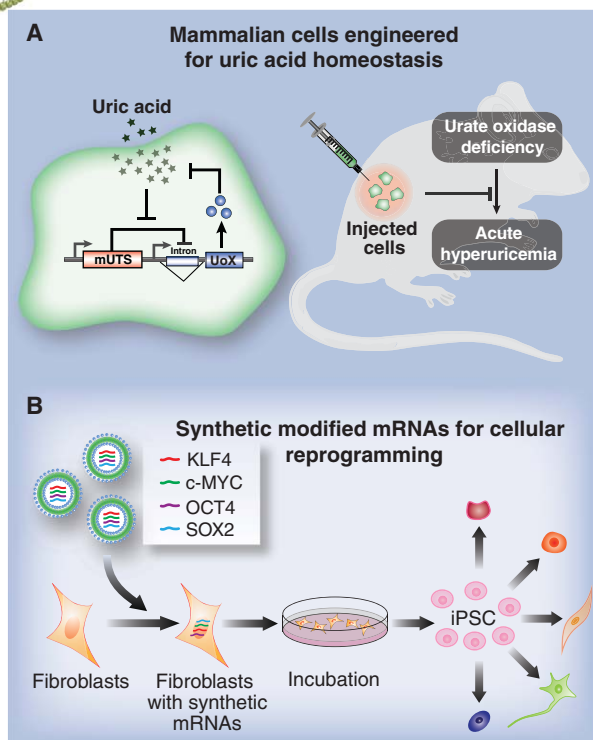


Fig. 4. Diseases can be targeted with new synthetic biology methods for cell therapy and regenerative medicine. **(A)** Urate homeostasis was restored in vivo by the delivery of cells with a synthetic circuit. Uric acid induced the derepression of an engineered urate oxidase, which then lowered uric acid levels in mice. **(B)** Synthetic modified RNAs encoding the KMOS transcription factors were delivered to mammalian fibroblasts to induce pluripotency upon translation. The RNA-induced pluripotent stem cells could be driven down numerous cell lineages.

translational medicine. These and related thrusts will benefit from emerging efforts to integrate synthetic biology with systems biology (37, 38).

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These developments will aid in the understanding of potential immune responses to synthetic constructs in the body and help identify approaches to ameliorate such responses. These efforts will be critical for developing safe and effective synthetic biology therapies.

Ultimately, we envision synthetic constructs that can sense and seek out aberrant conditions, remediate clinical insult, and restore function. Clearly, there is much to do before synthetic biology can realize its full clinical potential, but the examples discussed here provide insight into the field's exciting potential for helping to prevent and treat disease.

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Supporting Online Material

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 Figs. S1 to S3
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PERSPECTIVE

Bottom-Up Synthetic Biology: Engineering in a Tinkerer's World

Petra Schwillé*

How synthetic can "synthetic biology" be? A literal interpretation of the name of this new life science discipline invokes expectations of the systematic construction of biological systems with cells being built module by module—from the bottom up. But can this possibly be achieved, taking into account the enormous complexity and redundancy of living systems, which distinguish them quite remarkably from design features that characterize human inventions? There are several recent developments in biology, in tight conjunction with quantitative disciplines, that may bring this literal perspective into the realm of the possible. However, such bottom-up engineering requires tools that were originally designed by nature's greatest tinkerer: evolution.

An important feature of "synthetic biology" is that it draws on expertise from diverse disciplines; however, these disciplines

have not converged on what the new field encompasses. Biotechnologists view it mainly as a new way to organize and structure the art of ge-

netic engineering. To help, synthetic biology enforces the traditional engineering concepts of modularity and standardization and adapts logical operator structures from information processing (1). Nevertheless, the assembly of new biological systems for a variety of applications is still carried out in an existing organism; for clinical examples, see the review by Ruder et al. [see (2)]. Perhaps a more daring view comes from chemists and physicists who take the words literally and focus on the construction of biological systems from the bottom up. They suggest that synthetic biology could follow the tracks of synthetic organic chemistry and open up a new understanding of biology (3). This is not to suggest that something as complex as a eukaryotic or even a prokaryotic cell—end

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