Industrial Biotechnology

5885 Hollis Street, 4th Floor, Emeryville, CA 94608
Phone: +1.510.871.3272 Fax: +1.510.245.2223

This material is based upon work supported by the National Science Foundation under Grant No. 1818248.

Industrial Biotechnology

Industrial Biotechnology focuses on technical challenges relevant to industrial use of synthetic biology and the establishment of the United States as a global leader in the bio-based economy. Applications of engineering biology in this sector focus on increasing market share of bio-based products by US companies through the use of synthetic biology approaches, making sustainable manufacturing processes cost-competitive, accelerating innovation and discovery with respect to making new products and technologies, and generating products at scales necessary for economic viability. The goal is to stimulate investment in infrastructure, streamline production, and advance our use of engineering biology to improve lives. Many of the Challenges, Aims, and Objectives of the roadmap for *Industrial Biotechnology* are influenced by the findings of *Industrialization of biology: A roadmap to accelerate the advanced manufacturing of chemicals.* Committee on Industrialization of Biology: A Roadmap to Accelerate the Advanced Manufacturing of Chemicals, Board on Chemical Sciences and Technology, Board on Life Sciences, Division on Earth and Life Studies, & National Research Council. (2015). Washington (DC): National Academies Press (US).

Societal Challenge 1: Enable next-generation production through sustainable, cost-competitive, flexible, and efficient manufacturing processes.

- Science/Engineering Aim 1: Better use of abundant, renewable substrates to make specialty chemicals via economically viable processes.
    - Engineering Biology Objective 1: Modular systems (such as enzymes, consortia, and cell-free systems) that can adapt to different feedstocks and be easily modified to produce different target chemicals.
        - Gene Editing, Synthesis, and Assembly Achievement:
            - Ability to edit genomes of microbial and fungal species that can rapidly degrade cellulosic biomass and other renewable feedstocks.
        - Biomolecule, Pathway, and Circuit Engineering Achievement:
            - Rapid design and production of custom enzymes and enzyme pathways.
            - Assembled sets of proteins that can completely degrade sustainable feedstocks.
            - Regulatory components (including sensors and networks) that program the system to adapt to the feedstock, intermediates, and side products.
        - Host and Consortia Engineering Achievement:
            - Engineered microbial consortia with predictable composition, dynamics and function, to feed off of sequential byproducts in an (almost) closed-loop system.
        - Data Integration, Modeling, and Automation Achievement:
            - Novel analytics tools to enable prediction and manipulation of holistic microbial ecosystem function by incorporating both biological and environmental data.

        - Analytical tools to determine and predict matching of organism, strain, or pathway with feedstock/substrate source for best productivity, yield, and lowest cost.
    - Engineering Biology Objective 2: New enzymes and cells that work synergistically to degrade biomass and process by-products, possibly in combination with new chemical innovations.
        - Gene Editing, Synthesis, and Assembly Achievement:
            - Ability to synthesize, edit, assemble, and deliver many genes and regulatory components in a single cell.
        - Biomolecule, Pathway, and Circuit Engineering Achievement:
            - Enzymes engineered to degrade renewable materials and process byproducts faster and more completely.
            - Metabolic or protein engineering approaches to enable complete use of all substrate components and byproducts.
        - Host and Consortia Engineering Achievement:
            - Increased protein secretion rates to enable on-demand enzyme synthesis and release.
            - Engineered microbial consortia with predictable composition, dynamics and function, to feed off of sequential byproducts in an (almost) closed-loop system.
        - Data Integration, Modeling, and Automation Achievement:
            - Prediction of protein and cell assemblies that will exhibit desired target production, considering both composition and how components are physically assembled.
            - Prediction and modeling of microbial consortia functioning, specifically synergistic pathways and byproduct recycling.
    - Engineering Biology Objective 3: Use of novel and lesser-used substrates/feedstocks for manufacturing processes that are more efficient and more environmentally-sustainable than currently available substrates. This engineering biology objective is aimed at developing new substrate and feedstock systems based solely on biotechnology industry needs, rather than utilizing offshoots of systems created for other purposes (such as farming or animal feeds).
        - Biomolecule, Pathway, and Circuit Engineering Achievement:
            - Improved methods for transformation of organisms with heterologous pathways.
        - Host and Consortia Engineering Achievement:
            - Ability to engineer currently used organisms and hosts to work well with feedstocks deemed promising from environmental and cost standpoints.
        - Data Integration, Modeling, and Automation Achievement:
            - Data analysis approaches combining sustainability analyses with strain/pathway/methodology product, yield, and efficiency to

determine promise of lesser-known feedstocks from both industrial-productivity and environmental-sustainability standpoints.
        - Modeling methods combining environmental and economic factors to determine the best ways to implement production of new feedstocks.
- Science/Engineering Aim 2: More efficient production of (bio)chemicals, bio-based products, and other specialty materials. The goal of this Aim is to lower energy usage, lower waste processing, and reduce water use in manufacturing and industrial settings.
    - Engineering Biology Objective 1: Implementation of computational approaches to assemble new systems that consider multiple facets of production. (Including feedstocks, target product, available facilities, biological component characteristics and limitations, among others.)
        - Gene Editing, Synthesis, and Assembly Achievement:
            - Ability to synthesize, edit, assemble, and deliver many genes and regulatory components in a single cell.
            - Ability to edit genomes of diverse hosts, including microbes, fungi, and protists.
        - Biomolecule, Pathway, and Circuit Engineering Achievement:
            - Rapid design and production of custom enzymes and enzyme pathways.
            - Metabolic or protein engineering approaches to enable complete use of all substrate components.
        - Host and Consortia Engineering Achievement:
            - Engineered microbial consortia with predictable composition, dynamics and function, to feed off of sequential byproducts in an (almost) closed-loop system.
            - Development of fast-growing variants of non-model production hosts.
        - Data Integration, Modeling, and Automation Achievement:
            - Data integration methodology and approaches to describe and compare system performance.
            - Design-of-experiments approaches to obtain required data to enable prediction.
            - Artificial intelligence and/or machine learning approaches to predict how systems should be assembled considering production goals and constraints.
    - Engineering Biology Objective 2: Enable a broader range of microorganisms to be used in traditional biomanufacturing industries to expand the scope of natural product discovery and production.
        - Gene Editing, Synthesis, and Assembly Achievement:
            - Ability to synthesize, edit, assemble, and deliver many genes and regulatory components in a single cell.

        - Ability to edit genomes of diverse hosts, including microbes, fungi, and protists.
    - Biomolecule, Pathway, and Circuit Engineering Achievement:
        - Rapid adaptation of enzymes to work in the context of different hosts.
    - Host and Consortia Engineering Achievement:
        - Development of fast-growing variants of non-model production hosts.
    - Data Integration, Modeling, and Automation Achievement:
        - Prediction of media components, additives, environmental conditions that promote growth of non-model production hosts from genomic data.
- Engineering Biology Objective 3: Experimental and computational approaches to increase growth rates, yield, and efficiency of production hosts.
    - Gene Editing, Synthesis, and Assembly Achievement:
        - Ability to synthesize, edit, assemble, and deliver many genes and regulatory components in a single cell.
        - Ability to edit genomes of diverse hosts, including microbes, fungi, and protists.
    - Biomolecule, Pathway, and Circuit Engineering Achievement:
        - Development of regulatory components that enable dynamic regulation to optimally balance growth and production, especially of toxic or high-energy-requiring products.
    - Host and Consortia Engineering Achievement:
        - Ability to engineer non-model production hosts with increased growth rates and improved yield and efficiency, especially under bioproduction conditions.
    - Data Integration, Modeling, and Automation Achievement:
        - Computational approaches to classify mutations found in slow-growing production hosts, to define if they are necessary or detrimental for cell growth and processing.
        - Automation approaches to screen new candidate hosts for fast growth and desired production rates.

- Science/Engineering Aim 3: Modular manufacturing to enable flexible, on-demand production of a range of target chemicals. The goal of this Aim is to reduce transportation costs of both feedstocks and products to improve economic feasibility, especially of lower-value chemicals like biofuels.
    - Engineering Biology Objective 1: Development of commercial systems for on-demand manufacturing of commodity and high-value chemicals. The ideal system will include on-demand capabilities for both upstream (production) and downstream (purification) elements of the process.
        - Gene Editing, Synthesis, and Assembly Achievement:
            - Ability to synthesize, edit, assemble, and deliver many genes and regulatory components in a single cell.
            - Ability to edit genomes of diverse hosts, including microbes, fungi, and protists.
        - Biomolecule, Pathway, and Circuit Engineering Achievement:
            - Assembled sets of proteins that can completely degrade sustainable feedstocks.
            - Regulatory components (including sensors and networks) that program the system to adapt to the feedstock, intermediates, and side products.
        - Host and Consortia Engineering Achievement:
            - Engineered microbial consortia with predictable composition, dynamics and function, to feed off of sequential byproducts in an (almost) closed-loop system.
        - Data Integration, Modeling, and Automation Achievement:
            - Novel analytics tools to enable prediction and manipulation of holistic microbial ecosystem function by incorporating both biological and environmental data.
            - Analytics tools and approaches to develop flexible manufacturing processes.
            - Widely adopted methods for defining reproducible workflows that can be used by cloud laboratories to embed protocols for implementation, characterization, and verification and validation of components, pathways/circuits, subsystems, cells, consortia, and multicellular organisms.
            - Modular field production facilities that can accommodate many manufacturing protocols.
            - Establish life cycle assessments to determine efficiency, sustainability, and feasibility of protocols and processes.

- Engineering Biology Objective 2: Develop hosts or consortia that can generate multiple products from a single process. The goal of this Objective is to generate product streams which can easily be separated, or modified, to produce different target products, such as by changing an environmental condition or one biological component.
  - Gene Editing, Synthesis, and Assembly Achievement:
    - Ability to synthesize, edit, assemble, and deliver many genes and regulatory components in a single cell.
    - Ability to edit genomes of diverse hosts, including microbes, fungi, and protists.
    - Community-level, metagenome editing.
  - Biomolecule, Pathway, and Circuit Engineering Achievement:
    - Assembled sets of proteins that can completely degrade sustainable feedstocks.
    - Regulatory components (including sensors and networks) that program the system to adapt to the feedstock, intermediates, and side products.
    - Regulatory components that allow the user to easily switch between different target products.
  - Host and Consortia Engineering Achievement:
    - Ability to exert tight control over pathways (such as through dynamic metabolic engineering) that are not being used in production.
    - Engineered microbial consortia with predictable composition, dynamics, and function, to feed off of sequential byproducts in an (almost) closed-loop system.
    - Adaptable hosts optimized for production of multiple products.
  - Data Integration, Modeling, and Automation Achievement:
    - Novel analytics tools to enable prediction and manipulation of holistic microbial ecosystem function by incorporating both biological and environmental data.
    - Modeling and analytics tools for building systems with multiple objectives and constraints.
- Engineering Biology Objective 3: Engineer off-the-shelf hosts and microbial communities that can rapidly adapt and produce a target product(s) at high yield and high concentration. The goal of this objective is to enable production hosts that can rapidly adapt to different feedstocks, culture conditions, or toxic products and do so in increasingly closed-loop systems.
  - Gene Editing, Synthesis, and Assembly Achievement:
    - Ability to synthesize, edit, assemble, and deliver many genes and regulatory components in a single cell.
    - Ability to edit genomes of diverse hosts, including microbes, fungi, and protists.

        - Community-level, metagenome editing.
    - Biomolecule, Pathway, and Circuit Engineering Achievement:
        - Regulatory components (including sensors and networks) that program the community to adapt to the feedstock, intermediates, and side products.
    - Host and Consortia Engineering Achievement:
        - Engineer fast growing organisms that can rely on a variety of feedstocks.
        - Engineered microbial consortia with predictable composition, dynamics, and function, to feed off of sequential byproducts in an (almost) closed-loop system.
        - Ability to exert tight control over pathways (such as through dynamic metabolic engineering) that are not being used in production.
        - Reliable strategies for microbial community assembly that promote desired community composition and high-levels of productivity.
        - Engineered host organisms that can be stored without freezing and easily shipped.
        - Engineered hosts that produce fewer (or no) toxic by-products.
    - Data Integration, Modeling, and Automation Achievement:
        - Novel analytics tools to enable prediction and manipulation of holistic microbial ecosystem function by incorporating both biological and environmental data.
        - Automation strategies for assessing community composition and function dynamically.
        - Modular field production facilities that can accommodate many manufacturing protocols.

Societal Challenge 2: Scalable production of novel and existing products that are more sustainable and economically- and environmentally-friendly.

- Science/Engineering Aim 1: Improved ability to identify and make commodity, specialty, and high value chemicals and materials.
    - Engineering Biology Objective 1: Modeling, design, and test of pathways to make molecules and products that do not exist in nature.
        - Gene Editing, Synthesis, and Assembly Achievement:
            - Precise and automated generation of large collections of gene variants for pathway designs.
        - Biomolecule, Pathway, and Circuit Engineering Achievement:
            - Design of enzymes for converting unnatural substrates.
            - At-will design of non-natural pathways for the production of novel products and materials.

        - Host and Consortia Engineering Achievement:
            - Creation of hosts suitable for the production of certain natural and synthetic molecule families.
            - Pathway integration processes for hosts that are easy and high-throughput.
            - Understanding of, and solutions for, product toxicity to the host.
        - Data Integration, Modeling, and Automation Achievement:
            - At-will design of non-natural pathways for the production of novel products and materials.
            - Automated robotic screening of desired phenotypes coupled with precise analytics of desired molecules and side products.
    - Engineering Biology Objective 2: Novel methodologies for discovery and optimization of existing metabolic pathways in host organisms.
        - Gene Editing, Synthesis, and Assembly Achievement:
            - Precise and automated generation of large collections of gene variants.
            - Gene editing capabilities for diverse production organisms.
        - Biomolecule, Pathway, and Circuit Engineering Achievement:
            - Better enzyme and pathway design in industrial production hosts.
        - Host and Consortia Engineering Achievement:
            - Molecular tools for novel or unnatural production organisms.
        - Data Integration, Modeling, and Automation Achievement:
            - Automated robotic screening of desired phenotypes coupled with precise analytics of desired molecules and side products.
            - Accurate prediction of gene and enzyme function for poorly annotated genomes to allow more effective bioprospecting.
    - Engineering Biology Objective 3: Production of biologics in organisms at economically-viable scales.
        - Gene Editing, Synthesis, and Assembly Achievement:
            - Gene editing capabilities for diverse production organisms to ensure access to “the right tool for the job”.
        - Biomolecule, Pathway, and Circuit Engineering Achievement:
            - Novel biosensors that respond to cheaper and/or more efficient inducer molecules (chemical pathway “on/off switches”).
            - Automated directed evolution of multi-gene pathways to make a single host species or strain as productive as possible (such as able to synthesize multiple products).
            - Engineer cell consortia for orchestrated production of multiple products simultaneously.
        - Host and Consortia Engineering Achievement:
            - Engineer host organisms that grow quickly during production and more efficiently use diverse feedstocks.

        - Conversion of side products to valuable commodities for enhancing economic value.
    - Data Integration, Modeling, and Automation Achievement:
        - Improved prediction and analysis of flux via metabolic pathways to determine maximum theoretical yields under different fermentation conditions.
- Engineering Biology Objective 4: Commercialization of new types of products using bio-inspired fabrication, including engineered living materials and devices.
    - Gene Editing, Synthesis, and Assembly Achievement:
        - Precision gene editing *in vivo*.
        - Reliable and efficient delivery vectors for gene editing agents.
    - Biomolecule, Pathway, and Circuit Engineering Achievement:
        - Optimize pathways for the production of novel bio-polymer materials.
    - Host and Consortia Engineering Achievement:
        - Create engineered environments with three-dimensional structures and multiple cellular types ordered and arranged in a controlled manner throughout the structure.
        - Engineer hosts with controllable “kill switches”, decreased toxic byproducts, and decreased immunogenicity (especially for biosensors for multicellular hosts and/or environments).
    - Data Integration, Modeling, and Automation Achievement:
        - Enhanced prediction of compatibility between bio-fabricated materials and hosts/environments.

- Science/Engineering Aim 2: Manufacturing of consumable and infrastructure products via synthetic biology, including food, textiles, building materials, and packaging.
    - Engineering Biology Objective 1: Innovations in bio-based manufacturing of commodity products.
        - Gene Editing, Synthesis, and Assembly Achievement:
            - Highly efficient organismal gene and genome editing.
        - Biomolecule, Pathway, and Circuit Engineering Achievement:
            - Enable more efficient and rapid engineering of orthogonal chemical communications pathways between cells.
            - Heterologous pathway transformation into organisms from different kingdoms.
        - Host and Consortia Engineering Achievement:
            - General molecular toolbox that can be applied to a wide range of hosts.
        - Data Integration, Modeling, and Automation Achievement:
            - Screening capabilities for bio-based production.
            - Databases and foundries for microbial isolates.

    - Engineering Biology Objective 2: Synthetic and natural organisms that can convert agricultural wastes into commodity products.
        - Gene Editing, Synthesis, and Assembly Achievement:
            - Ability to synthesize, edit, assemble, and deliver many genes and regulatory components in a single cell.
            - Ability to edit genomes of diverse hosts, including microbes, fungi, and protists to find hosts that are naturally more efficient or that have more efficient pathways that can be placed into heterologous hosts.
        - Biomolecule, Pathway, and Circuit Engineering Achievement:
            - Engineered proteins and large complexes with efficient catalytic capabilities for conversion of bulk agricultural waste into products with downstream applicability.
            - Regulatory components (including sensors and networks) that program the organism to adapt to the feedstock, intermediates, and side products.
        - Host and Consortia Engineering Achievement:
            - General molecular toolbox that can be applied to a wide range of hosts.
            - Engineer hosts that can sustain production yield and efficiency under a wide range of stress conditions.
        - Data Integration, Modeling, and Automation Achievement:
            - Screening capabilities for bio-based production.
            - Libraries and foundries for microbial isolates.
            - Analysis and prediction of metagenomic data.
- Science/Engineering Aim 3: Safer production processes through reduced toxin use and toxic byproduct synthesis. (Including a reduction in downstream toxins from degradation of materials after use.)
    - Engineering Biology Objective 1: Risk, safety, and life-cycle assessments for bio-based manufacturing processes.
        - Gene Editing, Synthesis, and Assembly Achievement:
            - Safe, reliable, and efficient delivery vectors for gene editing agents.
        - Biomolecule, Pathway, and Circuit Engineering Achievement:
            - Engineered biosensors for toxic compounds.
        - Host and Consortia Engineering Achievement:
            - Metabolic pathway engineering to prevent synthesis of toxic byproducts and/or to metabolize toxins.
        - Data Integration, Modeling, and Automation Achievement:
            - High-throughput screening approaches to assess toxin production.
            - Development of holistic models of risk/safety with respect to manufacturing process (including employees), environment, and consumers.

        - Develop new and better life cycle assessment models for biomolecule production.
    - Engineering Biology Objective 2: Incentives and regulations, when needed, to ensure the safety of those involved in production and of consumers, as well as overall process sustainability.
        - Data Integration, Modeling, and Automation Achievement:
            - Development of holistic models of risk/safety with respect to manufacturing process (including employees), environment, and consumers.
            - Undertake life cycle assessment to determine efficiency, sustainability, and feasibility of protocols and processes.
    - Engineering Biology Objective 3: Engineer processes, pathways, and enzymes to minimize waste production.
        - Gene Editing, Synthesis, and Assembly Achievement:
            - Ability to synthesize, edit, assemble, and deliver many genes and regulatory components in a single cell.
            - Ability to edit genomes of diverse hosts, including microbes, fungi, and protists.
        - Biomolecule, Pathway, and Circuit Engineering Achievement:
            - Assembled sets of proteins that can completely degrade sustainable feedstocks.
            - Regulatory components (including sensors and networks) that program the system to adapt to the feedstock, intermediates, and side products.
        - Host and Consortia Engineering Achievement:
            - Engineered microbial consortia with predictable composition, dynamics, and function.
            - Metabolic pathway engineering to prevent synthesis of toxic byproducts and/or to metabolize toxins.
        - Data Integration, Modeling, and Automation Achievement:
            - Novel analytics tools to enable prediction and manipulation of holistic microbial ecosystem function by incorporating both biological and environmental data.
- Science/Engineering Aim 4: Better tools for rapidly translating desired products or process features into industrial workflows and accelerate time-to-market.
    - Engineering Biology Objective 1: Predictive and generalized models that allow laboratory-scale results to be accurately projected to industrial scale processes and vice-versa.
        - Gene Editing, Synthesis, and Assembly Achievement:
            - Methods for creating variant libraries that can be used for validating models of genetic circuits and pathways.
        - Biomolecule, Pathway, and Circuit Engineering Achievement:
            - Ability to predict function from sequence.

        - Ability to design sequences for desired functions.
    - Host and Consortia Engineering Achievement:
        - Better methods for predicting interactions between components and for designing components and subsystems that behave as expected.
    - Data Integration, Modeling, and Automation Achievement:
        - Higher-throughput data collection and analysis.
        - Better tools for predictive modeling across scales and environments.
        - Ability to estimate robustness of circuits and pathways to genetic, host, and environmental context.
- Engineering Biology Objective 2: Common (and reproducible) standards for biological components and subsystems that enable re-use and efficient component suppliers.
    - Gene Editing, Synthesis, and Assembly Achievement:
        - Methods for modular assembly of subsystems and replacement of components required to reconfigure subsystem interfaces.
    - Biomolecule, Pathway, and Circuit Engineering Achievement:
        - Protein libraries that allow modular replacement of domains to mix and match functions required for circuit and pathway engineering.
    - Host and Consortia Engineering Achievement:
        - Methods of defining “modules”, and flexible interconnection of modules, that maintain the desired function of the module independent of the operation of other parts of the circuit or genetic, host, environmental context.
    - Data Integration, Modeling, and Automation Achievement:
        - Methods for modeling components/subsystems that allows better characterization and prediction of effects of interaction with other modules and cellular resources, as well as the effects of uncertainty (such as context or system noise).
        - Automated techniques for assembly and characterization of complex circuits consisting of thousands of individual elements (organized as interacting subsystems).
- Engineering Biology Objective 3: Increased rate of design-build-test-learn cycles that combine design, modeling, prototyping, implementing, and characterization of components, pathways/circuits, subsystems, cells, consortia, and multicellular organisms.
    - Gene Editing, Synthesis, and Assembly Achievement:
        - Faster, lower-cost methods for creating genome-length sequences that are generated by design tools.

- Biomolecule, Pathway, and Circuit Engineering Achievement:
  - Better methods for design and characterization of proteins and multi-protein complexes for performing a wide variety of functions required at component to subsystem scales.
- Host and Consortia Engineering Achievement:
  - Ability to design, implement, and characterize circuits/pathways, subsystems, cells, consortia and multicellular organisms consisting of hundreds to millions of individual components through a modular, hierarchical framework that enables reuse of interacting components.
- Data Integration, Modeling, and Automation Achievement:
  - Layered and modular design abstractions and the modeling, characterization, and testing tools required to support the creation and use of components, pathways/circuits, and subsystems to create engineered cells, consortia, and multicellular organisms.

- Engineering Biology Objective 4: Deployment and improved use of automation for both research and translational activities to increase throughput.
  - Gene Editing, Synthesis, and Assembly Achievement:
    - Well-defined libraries with associated assembly and editing methods for genomic sequences that support the output of compilers and other design tools.
    - Increased cross-talk between geneticists and automation experts to hone efficient, high-throughput, automated laboratory protocols and workflows for gene editing and assembly and genetic library creation and exploration.
  - Biomolecule, Pathway, and Circuit Engineering Achievement:
    - Well-defined libraries with associated assembly and editing methods for protein domains and molecular machines that support the output of compilers and other design tools.
  - Host and Consortia Engineering Achievement:
    - Engineered hosts with predictable composition, dynamics, and function.
    - Continually refined physical automation infrastructure and processes (including factories, robots, assembly lines, and workflows) to enable more efficient and modular high-throughput engineering of multiple different kinds of organisms, pathways, and product outputs.
  - Data Integration, Modeling, and Automation Achievement:
    - Widely adopted methods for defining reproducible workflows that can be used by cloud laboratories to implement protocols for implementation, characterization, and verification and validation of components, pathways/circuits, sub-systems, cells, multicellular organisms, consortia, and automation platforms.

References

Alford, R. F., Leaver-Fay, A., Jeliazkov, J. R., O'Meara, M. J., DiMaio, F. P., Park, H., Shapovalov MV, Renfrew PD, Mulligan VK, Kappel K, Labonte JW, Pacella MS, Bonneau R, Bradley P, Dunbrack RL, Das R, Baker D, Kuhlman B, Kortemme T, Gray, J. J. (2017). The Rosetta All-Atom Energy Function for Macromolecular Modeling and Design. *Journal of Chemical Theory and Computation*, *13*(6), 3031–3048. https://doi.org/10.1021/acs.jctc.7b00125

Ali, H., & Khan, E. (2018). Trophic transfer, bioaccumulation, and biomagnification of non-essential hazardous heavy metals and metalloids in food chains/webs—Concepts and implications for wildlife and human health. *Human and Ecological Risk Assessment: An International Journal*, 1–24. https://doi.org/10.1080/10807039.2018.1469398

AlQuraishi, M. (2019). AlphaFold at CASP13. *Bioinformatics*. https://doi.org/10.1093/bioinformatics/btz422

Badran, A. H., & Liu, D. R. (2015). In vivo continuous directed evolution. *Current Opinion in Chemical Biology*, *24*, 1–10. https://doi.org/10.1016/j.cbpa.2014.09.040

Bar-Even, A., Noor, E., Savir, Y., Liebermeister, W., Davidi, D., Tawfik, D. S., & Milo, R. (2011). The moderately efficient enzyme: evolutionary and physicochemical trends shaping enzyme parameters. *Biochemistry*, *50*(21), 4402–4410. https://doi.org/10.1021/bi2002289

Bier, E., Harrison, M. M., O'Connor-Giles, K. M., & Wildonger, J. (2018). Advances in Engineering the Fly Genome with the CRISPR-Cas System. *Genetics*, *208*(1), 1–18. https://doi.org/10.1534/genetics.117.1113

Blind, M., & Blank, M. (2015). Aptamer selection technology and recent advances. *Molecular Therapy. Nucleic Acids*, *4*, e223. https://doi.org/10.1038/mtna.2014.74

Boeing, P., Leon, M., Nesbeth, D. N., Finkelstein, A., & Barnes, C. P. (2018). Towards an Aspect-Oriented Design and Modelling Framework for Synthetic Biology. *Processes (Basel, Switzerland)*, *6*(9), 167. https://doi.org/10.3390/pr6090167

Cambray, G., Guimaraes, J. C., & Arkin, A. P. (2018). Evaluation of 244,000 synthetic sequences reveals design principles to optimize translation in Escherichia coli. *Nature Biotechnology*, *36*(10), 1005–1015. https://doi.org/10.1038/nbt.4238

Carlson, P. D., & Lucks, J. B. (2019). Elements of RNA design. *Biochemistry*, *58*(11), 1457–1459. https://doi.org/10.1021/acs.biochem.8b01129

Carothers, J. M., Goler, J. A., Juminaga, D., & Keasling, J. D. (2011). Model-driven engineering of RNA devices to quantitatively program gene expression. *Science*, *334*(6063), 1716–1719. https://doi.org/10.1126/science.1212209

Carothers, J. M., Oestreich, S. C., Davis, J. H., & Szostak, J. W. (2004). Informational complexity and functional activity of RNA structures. *Journal of the American Chemical Society*, *126*(16), 5130–5137. https://doi.org/10.1021/ja031504a

Chappell, J., Westbrook, A., Verosloff, M., & Lucks, J. B. (2017). Computational design of small transcription activating RNAs for versatile and dynamic gene regulation. *Nature Communications*, *8*(1), 1051. https://doi.org/10.1038/s41467-017-01082-6

Cherry, K. M., & Qian, L. (2018). Scaling up molecular pattern recognition with DNA-based winner-take-all neural networks. *Nature*, *559*(7714), 370–376. https://doi.org/10.1038/s41586-018-0289-6

Clark, D. S., & Blanch, H. W. (1997). *Biochemical Engineering (Chemical Industries)* (2nd ed., p. 716). New York, New York: Crc Press.

Costello, Z., & Martin, H. G. (2018). A machine learning approach to predict metabolic pathway dynamics from time-series multiomics data. *Npj Systems Biology and Applications*, *4*, 19. https://doi.org/10.1038/s41540-018-0054-3

Cox, J. C., Hayhurst, A., Hesselberth, J., Bayer, T. S., Georgiou, G., & Ellington, A. D. (2002). Automated selection of aptamers against protein targets translated in vitro: from gene to aptamer. *Nucleic Acids Research*, *30*(20), e108. https://doi.org/10.1093/nar/gnf107

Cuperus, J. T., Groves, B., Kuchina, A., Rosenberg, A. B., Jojic, N., Fields, S., & Seelig, G. (2017). Deep learning of the regulatory grammar of yeast 5' untranslated regions from 500,000 random sequences. *Genome Research*, *27*(12), 2015–2024. https://doi.org/10.1101/gr.224964.117

Das, R., Karanicolas, J., & Baker, D. (2010). Atomic accuracy in predicting and designing noncanonical RNA structure. *Nature Methods*, *7*(4), 291–294. https://doi.org/10.1038/nmeth.1433

Davey, J. A., Damry, A. M., Goto, N. K., & Chica, R. A. (2017). Rational design of proteins that exchange on functional timescales. *Nature Chemical Biology*, *13*(12), 1280–1285. https://doi.org/10.1038/nchembio.2503

de Kok, S., Stanton, L. H., Slaby, T., Durot, M., Holmes, V. F., Patel, K. G., Platt D, Shapland EB, Serber Z, Dean J, Newman JD, Chandran, S. S. (2014). Rapid and reliable DNA assembly via ligase cycling reaction. *ACS Synthetic Biology*, *3*(2), 97–106. https://doi.org/10.1021/sb4001992

Dehingia, M., Adak, A., & Khan, M. R. (2019). Ethnicity-Influenced Microbiota: A Future Healthcare Perspective. *Trends in Microbiology*, *27*(3), 191–193. https://doi.org/10.1016/j.tim.2019.01.002

DeLoache, W. C., Russ, Z. N., & Dueber, J. E. (2016). Towards repurposing the yeast peroxisome for compartmentalizing heterologous metabolic pathways. *Nature Communications*, *7*, 11152. https://doi.org/10.1038/ncomms11152

Doudna, J. A., & Charpentier, E. (2014). Genome editing. The new frontier of genome engineering with CRISPR-Cas9. *Science*, *346*(6213), 1258096. https://doi.org/10.1126/science.1258096

Ellington, A. D., & Szostak, J. W. (1990). In vitro selection of RNA molecules that bind specific ligands. *Nature*, *346*(6287), 818–822. https://doi.org/10.1038/346818a0

Engler, C., Kandzia, R., & Marillonnet, S. (2008). A one pot, one step, precision cloning method with high throughput capability. *Plos One*, *3*(11), e3647. https://doi.org/10.1371/journal.pone.0003647

Espah Borujeni, A., Mishler, D. M., Wang, J., Huso, W., & Salis, H. M. (2016). Automated physics-based design of synthetic riboswitches from diverse RNA aptamers. *Nucleic Acids Research*, *44*(1), 1–13. https://doi.org/10.1093/nar/gkv1289

Espah Borujeni, A., & Salis, H. M. (2016). Translation initiation is controlled by RNA folding kinetics via a ribosome drafting mechanism. *Journal of the American Chemical Society*, *138*(22), 7016–7023. https://doi.org/10.1021/jacs.6b01453

Esvelt, K. M., Carlson, J. C., & Liu, D. R. (2011). A system for the continuous directed evolution of biomolecules. *Nature*, *472*(7344), 499–503. https://doi.org/10.1038/nature09929

Fan, W., Guo, Q., Liu, C., Liu, X., Zhang, M., Long, D., Xiang, Z., Zhao, A. (2018). Two mulberry phytochelatin synthase genes confer zinc/cadmium tolerance and accumulation in transgenic Arabidopsis and tobacco. *Gene*, *645*, 95–104. https://doi.org/10.1016/j.gene.2017.12.042

Farré, G., Blancquaert, D., Capell, T., Van Der Straeten, D., Christou, P., & Zhu, C. (2014). Engineering complex metabolic pathways in plants. *Annual Review of Plant Biology*, *65*, 187–223. https://doi.org/10.1146/annurev-arplant-050213-035825

Galdzicki, M., Clancy, K. P., Oberortner, E., Pocock, M., Quinn, J. Y., Rodriguez, C. A., Roehner N, Wilson ML, Adam L, Anderson JC, Bartley BA, Beal J, Chandran D, Chen J, Densmore D, Endy D, Grünberg R, Hallinan J, Hillson NJ, Johnson JD, Kuchinsky A, Lux M, Misirli G, Peccoud J, Plahar HA, Sirin E, Stan GB, Villalobos A, Wipat A, Gennari JH, Myers CJ, Sauro, H. M. (2014). The Synthetic Biology Open Language (SBOL) provides a community standard for communicating designs in synthetic biology. *Nature Biotechnology*, *32*(6), 545–550. https://doi.org/10.1038/nbt.2891

Gantz, V. M., & Bier, E. (2015). Genome editing. The mutagenic chain reaction: a method for converting heterozygous to homozygous mutations. *Science*, *348*(6233), 442–444. https://doi.org/10.1126/science.aaa5945

Gantz, V. M., Jasinskiene, N., Tatarenkova, O., Fazekas, A., Macias, V. M., Bier, E., & James, A. A. (2015). Highly efficient Cas9-mediated gene drive for population modification of the malaria vector mosquito Anopheles stephensi. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(49), E6736–43. https://doi.org/10.1073/pnas.1521077112

Gibson, D. G. (2011). Enzymatic assembly of overlapping DNA fragments. *Methods in Enzymology*, *498*, 349–361. https://doi.org/10.1016/B978-0-12-385120-8.00015-2

Gibson, D. G., Young, L., Chuang, R.-Y., Venter, J. C., Hutchison, C. A., & Smith, H. O. (2009). Enzymatic assembly of DNA molecules up to several hundred kilobases. *Nature Methods*, *6*(5), 343–345. https://doi.org/10.1038/nmeth.1318

Gilbert, J. A., & Melton, L. (2018). Verily project releases millions of factory-reared mosquitoes. *Nature Biotechnology*, *36*(9), 781–782. https://doi.org/10.1038/nbt0918-781a

Gilbert, L. A., Larson, M. H., Morsut, L., Liu, Z., Brar, G. A., Torres, S. E., Stern-Ginossar N, Brandman O, Whitehead EH, Doudna JA, Lim WA, Weissman JS, Qi, L. S. (2013). CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes. *Cell*, *154*(2), 442–451. https://doi.org/10.1016/j.cell.2013.06.044

Goldsmith, M., & Tawfik, D. S. (2017). Enzyme engineering: reaching the maximal catalytic efficiency peak. *Current Opinion in Structural Biology*, *47*, 140–150. https://doi.org/10.1016/j.sbi.2017.09.002

Goodwin, S., McPherson, J. D., & McCombie, W. R. (2016). Coming of age: ten years of next-generation sequencing technologies. *Nature Reviews. Genetics*, *17*(6), 333–351. https://doi.org/10.1038/nrg.2016.49

Green, A. A., Silver, P. A., Collins, J. J., & Yin, P. (2014). Toehold switches: de-novo-designed regulators of gene expression. *Cell*, *159*(4), 925–939. https://doi.org/10.1016/j.cell.2014.10.002

Grunwald, H. A., Gantz, V. M., Poplawski, G., Xu, X.-R. S., Bier, E., & Cooper, K. L. (2019). Super-Mendelian inheritance mediated by CRISPR-Cas9 in the female mouse germline. *Nature*, *566*(7742), 105–109. https://doi.org/10.1038/s41586-019-0875-2

Haitjema, C. H., Solomon, K. V., Henske, J. K., Theodorou, M. K., & O'Malley, M. A. (2014). Anaerobic gut fungi: Advances in isolation, culture, and cellulolytic enzyme discovery for biofuel production. *Biotechnology and Bioengineering*, *111*(8), 1471–1482. https://doi.org/10.1002/bit.25264

Halperin, S. O., Tou, C. J., Wong, E. B., Modavi, C., Schaffer, D. V., & Dueber, J. E. (2018). CRISPR-guided DNA polymerases enable diversification of all nucleotides in a tunable window. *Nature*, *560*(7717), 248–252. https://doi.org/10.1038/s41586-018-0384-8

Ham, T. S., Dmytriv, Z., Plahar, H., Chen, J., Hillson, N. J., & Keasling, J. D. (2012). Design, implementation and practice of JBEI-ICE: an open source biological part registry platform and tools. *Nucleic Acids Research*, *40*(18), e141. https://doi.org/10.1093/nar/gks531

Heirendt, L., Arreckx, S., Pfau, T., Mendoza, S. N., Richelle, A., Heinken, A., Haraldsdóttir HS, Wachowiak J, Keating SM, Vlasov V, Magnusdóttir S, Ng CY, Preciat G, Žagare A, Chan SHJ, Aurich MK, Clancy CM, Modamio J, Sauls JT, Noronha A, Bordbar A, Cousins B, El Assal DC, Valcarcel LV, Apaolaza I, Ghaderi S, Ahookhosh M, Ben Guebila M, Kostromins A, Sompairac N, Le HM, Ma D, Sun Y, Wang L, Yurkovich JT, Oliveira MAP, Vuong PT, El Assal LP, Kuperstein I, Zinovyev A, Hinton HS, Bryant WA, Aragón Artacho FJ, Planes FJ, Stalidzans E, Maass A, Vempala S, Hucka M, Saunders MA, Maranas CD, Lewis NE, Sauter T, Palsson BØ, Thiele I, Vlasov, V. (2019). Creation and analysis of biochemical constraint-based models using the COBRA Toolbox v.3.0. *Nature Protocols*, *14*(3), 639–702. https://doi.org/10.1038/s41596-018-0098-2

Higgins, S. A., & Savage, D. F. (2018). Protein science by DNA sequencing: how advances in molecular biology are accelerating biochemistry. *Biochemistry*, *57*(1), 38–46. https://doi.org/10.1021/acs.biochem.7b00886

Hillson, N., Caddick, M., Cai, Y., Carrasco, J. A., Chang, M. W., Curach, N. C., Bell DJ, Le Feuvre R, Friedman DC, Fu X, Gold ND, Herrgård MJ, Holowko MB, Johnson JR, Johnson RA, Keasling JD, Kitney RI, Kondo A, Liu C, Martin VJJ, Menolascina F, Ogino C, Patron NJ, Pavan M, Poh CL, Pretorius IS, Rosser SJ, Scrutton NS, Storch M, Tekotte H, Travnik E, Vickers CE, Yew WS, Yuan Y, Zhao H, Freemont, P. S. (2019). Building a global alliance of biofoundries. *Nature Communications*, *10*(1), 2040. https://doi.org/10.1038/s41467-019-10079-2

Hooker, S. E., Woods-Burnham, L., Bathina, M., Lloyd, S. M., Gorjala, P., Mitra, R., Nonn L, Kimbro KS, Kittles, R. (2019). Genetic ancestry analysis reveals misclassification of commonly used cancer cell lines. *Cancer Epidemiology, Biomarkers & Prevention*. https://doi.org/10.1158/1055-9965.EPI-18-1132

Hoshika, S., Leal, N. A., Kim, M.-J., Kim, M.-S., Karalkar, N. B., Kim, H.-J., Bates AM, Watkins NE, SantaLucia HA, Meyer AJ, DasGupta S, Piccirilli JA, Ellington AD, SantaLucia J, Georgiadis MM, Benner, S. A. (2019). Hachimoji DNA and RNA: A genetic system with eight building blocks. *Science*, *363*(6429), 884–887. https://doi.org/10.1126/science.aat0971

Hsiao, V., Cheng, A., & Murray, R. M. (2016). *Design and application of stationary phase combinatorial promoters*. MurrayWiki. Retrieved from http://www.cds.caltech.edu/~murray/preprints/hcm16-seed_s.pdf

Huang, A., Nguyen, P. Q., Stark, J. C., Takahashi, M. K., Donghia, N., Ferrante, T., Dy AJ, Hsu KJ, Dubner RS, Pardee K, Jewett MC, Collins, J. J. (2018). BioBits™ Explorer: A modular synthetic biology education kit. *Science Advances*, *4*(8), eaat5105. https://doi.org/10.1126/sciadv.aat5105

Hughes, R. A., & Ellington, A. D. (2017). Synthetic DNA synthesis and assembly: putting the synthetic in synthetic biology. *Cold Spring Harbor Perspectives in Biology*, *9*(1). https://doi.org/10.1101/cshperspect.a023812

Jakobson, C. M., Chen, Y., Slininger, M. F., Valdivia, E., Kim, E. Y., & Tullman-Ercek, D. (2016). Tuning the catalytic activity of subcellular nanoreactors. *Journal of Molecular Biology*, *428*(15), 2989–2996. https://doi.org/10.1016/j.jmb.2016.07.006

Jeske, L., Placzek, S., Schomburg, I., Chang, A., & Schomburg, D. (2019). BRENDA in 2019: a European ELIXIR core data resource. *Nucleic Acids Research*, *47*(D1), D542–D549. https://doi.org/10.1093/nar/gky1048

Johns, N. I., Gomes, A. L. C., Yim, S. S., Yang, A., Blazejewski, T., Smillie, C. S., Smith MB, Alm EJ, Kosuri S, Wang, H. H. (2018). Metagenomic mining of regulatory elements enables programmable species-selective gene expression. *Nature Methods*, *15*(5), 323–329. https://doi.org/10.1038/nmeth.4633

Jones, H. P., Holmes, N. D., Butchart, S. H. M., Tershy, B. R., Kappes, P. J., Corkery, I., Aguirre-Muñoz A, Armstrong DP, Bonnaud E, Burbidge AA, Campbell K, Courchamp F, Cowan PE, Cuthbert RJ, Ebbert S, Genovesi P, Howald GR, Keitt BS, Kress SW, Miskelly CM, Oppel S, Poncet S, Rauzon MJ, Rocamora G, Russell JC, Samaniego-Herrera A, Seddon PJ, Spatz DR, Towns DR, Croll, D. A. (2016). Invasive mammal eradication on islands results in substantial conservation gains. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(15), 4033–4038. https://doi.org/10.1073/pnas.1521179113

Karim, A. S., & Jewett, M. C. (2016). A cell-free framework for rapid biosynthetic pathway prototyping and enzyme discovery. *Metabolic Engineering*, *36*, 116–126. https://doi.org/10.1016/j.ymben.2016.03.002

Kedzierska, K., Valkenburg, S. A., Doherty, P. C., Davenport, M. P., & Venturi, V. (2012). Use it or lose it: establishment and persistence of T cell memory. *Frontiers in Immunology*, *3*, 357. https://doi.org/10.3389/fimmu.2012.00357

Kim, E. Y., & Tullman-Ercek, D. (2014). A rapid flow cytometry assay for the relative quantification of protein encapsulation into bacterial microcompartments. *Biotechnology Journal*, *9*(3), 348–354. https://doi.org/10.1002/biot.201300391

Kong, W., Meldgin, D. R., Collins, J. J., & Lu, T. (2018). Designing microbial consortia with defined social interactions. *Nature Chemical Biology*, *14*(8), 821–829. https://doi.org/10.1038/s41589-018-0091-7

Kosuri, S., & Church, G. M. (2014). Large-scale de novo DNA synthesis: technologies and applications. *Nature Methods*, *11*(5), 499–507. https://doi.org/10.1038/nmeth.2918

Kuo-chen, C., & Shou-ping, J. (1974). Studies on the rate of diffusion-controlled reactions of enzymes. Spatial factor and force field factor. *Scientia Sinica*, *27*(5), 664–680.

Kyrou, K., Hammond, A. M., Galizi, R., Kranjc, N., Burt, A., Beaghton, A. K., Nolan, T., Crisanti, A. (2018). A CRISPR-Cas9 gene drive targeting doublesex causes complete population suppression in caged Anopheles gambiae mosquitoes. *Nature Biotechnology*, *36*(11), 1062–1066. https://doi.org/10.1038/nbt.4245

Lane, R. S., & Quistad, G. B. (1998). Borreliacidal Factor in the Blood of the Western Fence Lizard (Sceloporus occidentalis). *The Journal of Parasitology*, *84*(1), 29. https://doi.org/10.2307/3284524

Leistra, A. N., Amador, P., Buvanendiran, A., Moon-Walker, A., & Contreras, L. M. (2017). Rational modular RNA engineering based on in vivo profiling of structural accessibility. *ACS Synthetic Biology*, *6*(12), 2228–2240. https://doi.org/10.1021/acssynbio.7b00185

Leistra, A. N., Curtis, N. C., & Contreras, L. M. (2019). Regulatory non-coding sRNAs in bacterial metabolic pathway engineering. *Metabolic Engineering*, *52*, 190–214. https://doi.org/10.1016/j.ymben.2018.11.013

Li, M. Z., & Elledge, S. J. (2007). Harnessing homologous recombination in vitro to generate recombinant DNA via SLIC. *Nature Methods*, *4*(3), 251–256. https://doi.org/10.1038/nmeth1010

Linshiz, G., Stawski, N., Poust, S., Bi, C., Keasling, J. D., & Hillson, N. J. (2013). PaR-PaR laboratory automation platform. *ACS Synthetic Biology*, *2*(5), 216–222. https://doi.org/10.1021/sb300075t

Long, M. R., Ong, W. K., & Reed, J. L. (2015). Computational methods in metabolic engineering for strain design. *Current Opinion in Biotechnology*, *34*, 135–141. https://doi.org/10.1016/j.copbio.2014.12.019

Looi, F. Y., Baker, M. L., Townson, T., Richard, M., Novak, B., Doran, T. J., & Short, K. R. (2018). Creating disease resistant chickens: A viable solution to avian influenza? *Viruses*, *10*(10). https://doi.org/10.3390/v10100561

Lubertozzi, D., & Keasling, J. D. (2009). Developing Aspergillus as a host for heterologous expression. *Biotechnology Advances*, *27*(1), 53–75. https://doi.org/10.1016/j.biotechadv.2008.09.001

Ma, S., Saaem, I., & Tian, J. (2012). Error correction in gene synthesis technology. *Trends in Biotechnology*, 30(3), 147–154. https://doi.org/10.1016/j.tibtech.2011.10.002

Markley, A. L., Begemann, M. B., Clarke, R. E., Gordon, G. C., & Pfleger, B. F. (2015). Synthetic biology toolbox for controlling gene expression in the cyanobacterium Synechococcus sp. strain PCC 7002. *ACS Synthetic Biology*, *4*(5), 595–603. https://doi.org/10.1021/sb500260k

Martin, R. W., Des Soye, B. J., Kwon, Y.-C., Kay, J., Davis, R. G., Thomas, P. M., Majewska NI, Chen CX, Marcum RD, Weiss MG, Stoddart AE, Amiram M, Ranji Charna AK, Patel JR, Isaacs FJ, Kelleher NL, Hong SH, Jewett, M. C. (2018). Cell-free protein synthesis from genomically recoded bacteria enables multisite incorporation of noncanonical amino acids. *Nature Communications*, *9*(1), 1203. https://doi.org/10.1038/s41467-018-03469-5

McCarty, N. S., & Ledesma-Amaro, R. (2019). Synthetic biology tools to engineer microbial communities for biotechnology. *Trends in Biotechnology*, *37*(2), 181–197. https://doi.org/10.1016/j.tibtech.2018.11.002

McDermott, J., & Hardeman, M. (2018). Increasing Your Research's Exposure on Figshare Using the FAIR Data Principles. *Figshare*. https://doi.org/10.6084/m9.figshare.7429559.v2

Medema, M. H., van Raaphorst, R., Takano, E., & Breitling, R. (2012). Computational tools for the synthetic design of biochemical pathways. *Nature Reviews. Microbiology*, *10*(3), 191–202. https://doi.org/10.1038/nrmicro2717

Molteni, M. (2019, March 10). 23andMe's New Diabetes Test Has Experts Asking Who It's For | WIRED. Retrieved May 21, 2019, from https://www.wired.com/story/23andmes-new-diabetes-test-has-experts-asking-who-its-for/

Moore, S. J., MacDonald, J. T., Wienecke, S., Ishwarbhai, A., Tsipa, A., Aw, R., Kylilis N, Bell DJ, McClymont DW, Jensen K, Polizzi KM, Biedendieck R, Freemont, P. S. (2018). Rapid acquisition and model-based analysis of cell-free transcription-translation reactions from nonmodel bacteria. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(19), E4340–E4349. https://doi.org/10.1073/pnas.1715806115

Morrell, W. C., Birkel, G. W., Forrer, M., Lopez, T., Backman, T. W. H., Dussault, M., Petzold CJ, Baidoo EEK, Costello Z, Ando D, Alonso-Gutierrez J, George KW, Mukhopadhyay A, Vaino I, Keasling JD, Adams PD, Hillson NJ, Garcia Martin, H. (2017). The Experiment Data Depot: A Web-Based Software Tool for Biological Experimental Data Storage, Sharing, and Visualization. *ACS Synthetic Biology*, *6*(12), 2248–2259. https://doi.org/10.1021/acssynbio.7b00204

Muthusaravanan, S., Sivarajasekar, N., Vivek, J. S., Paramasivan, T., Naushad, M., Prakashmaran, J., Gayathri V, Al-Duaij, O. K. (2018). Phytoremediation of heavy metals: mechanisms, methods and enhancements. *Environmental Chemistry Letters*, *16*(4), 1–21. https://doi.org/10.1007/s10311-018-0762-3

Nahar, N., Rahman, A., Nawani, N. N., Ghosh, S., & Mandal, A. (2017). Phytoremediation of arsenic from the contaminated soil using transgenic tobacco plants expressing ACR2 gene of Arabidopsis thaliana. *Journal of Plant Physiology*, *218*, 121–126. https://doi.org/10.1016/j.jplph.2017.08.001

Naran, K., Nundalall, T., Chetty, S., & Barth, S. (2018). Principles of immunotherapy: implications for treatment strategies in cancer and infectious diseases. *Frontiers in Microbiology*, *9*, 3158. https://doi.org/10.3389/fmicb.2018.03158

National Academies of Sciences, Engineering, and Medicine, Division on Earth and Life Studies, Board on Life Sciences, Board on Chemical Sciences and Technology, & Committee on Strategies for Identifying and Addressing Potential Biodefense Vulnerabilities Posed by Synthetic Biology. (2018). *Biodefense in the age of synthetic biology*. Washington (DC): National Academies Press (US). https://doi.org/10.17226/24890

National Research Council (US) Committee on Assessing the Importance and Impact of Glycomics and Glycosciences. (2012). *Transforming glycoscience: A roadmap for the future*. Washington (DC): National Academies Press (US). https://doi.org/10.17226/13446

National Research Council (US) Committee on Industrialization of Biology: A Roadmap to Accelerate the Advanced Manufacturing of Chemicals, Board on Chemical Sciences and Technology, Board on Life Sciences, Division on Earth and Life Studies. (2015). *Industrialization of biology: A roadmap to accelerate the advanced manufacturing of chemicals.* Washington (DC): National Academies Press (US). https://doi.org/10.17226/19001

National Research Council (US) Committee on Scientific Evaluation of the Introduction of Genetically Modified Microorganisms and Plants into the Environment. (1989). *Field testing genetically modified organisms: framework for decisions*. Washington (DC): National Academies Press (US). https://doi.org/10.17226/1431

Nielsen, A. A. K., Der, B. S., Shin, J., Vaidyanathan, P., Paralanov, V., Strychalski, E. A., Ross D, Densmore D, Voigt, C. A. (2016). Genetic circuit design automation. *Science*, *352*(6281), aac7341. https://doi.org/10.1126/science.aac7341

Niu, D., Wei, H.-J., Lin, L., George, H., Wang, T., Lee, I.-H., Zhao HY, Wang Y, Kan Y, Shrock E, Lesha E, Wang G, Luo Y, Qing Y, Jiao D, Zhao H, Zhou X, Wang S, Wei H, Güell M, Church GM, Yang, L. (2017). Inactivation of porcine endogenous retrovirus in pigs using CRISPR-Cas9. *Science*, *357*(6357), 1303–1307. https://doi.org/10.1126/science.aan4187

Pacheco, A. R., Moel, M., & Segrè, D. (2019). Costless metabolic secretions as drivers of interspecies interactions in microbial ecosystems. *Nature Communications*, *10*(1), 103. https://doi.org/10.1038/s41467-018-07946-9

Palacino, J., Swalley, S. E., Song, C., Cheung, A. K., Shu, L., Zhang, X., Van Hoosear M, Shin Y, Chin DN, Keller CG, Beibel M, Renaud NA, Smith TM, Salcius M, Shi X, Hild M, Servais R, Jain M, Deng L, Bullock C, McLellan M, Schuierer S, Murphy L, Blommers MJ, Blaustein C, Berenshteyn F, Lacoste A, Thomas JR, Roma G, Michaud GA, Tseng BS, Porter JA, Myer VE, Tallarico JA, Hamann LG, Curtis D, Fishman MC, Dietrich WF, Dales NA, Sivasankaran, R. (2015). SMN2 splice modulators enhance U1-pre-mRNA association and rescue SMA mice. *Nature Chemical Biology*, *11*(7), 511–517. https://doi.org/10.1038/nchembio.1837

Palluk, S., Arlow, D. H., de Rond, T., Barthel, S., Kang, J. S., Bector, R., Baghdassarian HM, Truong AN, Kim PW, Singh AK, Hillson NJ, Keasling, J. D. (2018). De novo DNA synthesis using polymerase-nucleotide conjugates. *Nature Biotechnology*, *36*(7), 645–650. https://doi.org/10.1038/nbt.4173

Pardee, K., Green, A. A., Takahashi, M. K., Braff, D., Lambert, G., Lee, J. W., Ferrante T, Ma D, Donghia N, Fan M, Daringer NM, Bosch I, Dudley DM, O'Connor DH, Gehrke L, Collins, J. J. (2016). Rapid, Low-Cost Detection of Zika Virus Using Programmable Biomolecular Components. *Cell*, *165*(5), 1255–1266. https://doi.org/10.1016/j.cell.2016.04.059

Pearl, J. (2018). Theoretical Impediments to Machine Learning With Seven Sparks from the Causal Revolution. In *Proceedings of the Eleventh ACM International Conference on Web Search and Data Mining' ' - WSDM '18* (pp. 3–3). New York, New York, USA: ACM Press. https://doi.org/10.1145/3159652.3176182

Plesa, C., Sidore, A. M., Lubock, N. B., Zhang, D., & Kosuri, S. (2018). Multiplexed gene synthesis in emulsions for exploring protein functional landscapes. *Science*, *359*(6373), 343–347. https://doi.org/10.1126/science.aao5167

Qi, L. S., Larson, M. H., Gilbert, L. A., Doudna, J. A., Weissman, J. S., Arkin, A. P., & Lim, W. A. (2013). Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. *Cell*, *152*(5), 1173–1183. https://doi.org/10.1016/j.cell.2013.02.022

Qian, L., & Winfree, E. (2011). Scaling up digital circuit computation with DNA strand displacement cascades. *Science*, *332*(6034), 1196–1201. https://doi.org/10.1126/science.1200520

Rai, P. K., Lee, S. S., Zhang, M., Tsang, Y. F., & Kim, K.-H. (2019). Heavy metals in food crops: Health risks, fate, mechanisms, and management. *Environment International*, *125*, 365–385. https://doi.org/10.1016/j.envint.2019.01.067

Ravikumar, A., Arrieta, A., & Liu, C. C. (2014). An orthogonal DNA replication system in yeast. *Nature Chemical Biology*, *10*(3), 175–177. https://doi.org/10.1038/nchembio.1439

Ravikumar, A., Arzumanyan, G. A., Obadi, M. K. A., Javanpour, A. A., & Liu, C. C. (2018). Scalable, Continuous Evolution of Genes at Mutation Rates above Genomic Error Thresholds. *Cell*, *175*(7), 1946–1957.e13. https://doi.org/10.1016/j.cell.2018.10.021

Reva, B. A., Finkelstein, A. V., & Skolnick, J. (1998). What is the probability of a chance prediction of a protein structure with an rmsd of 6 A? *Folding & Design*, *3*(2), 141–147. https://doi.org/10.1016/S1359-0278(98)00019-4

Richardson, S. M., Mitchell, L. A., Stracquadanio, G., Yang, K., Dymond, J. S., DiCarlo, J. E., Lee D, Huang CL, Chandrasegaran S, Cai Y, Boeke JD, Bader, J. S. (2017). Design of a synthetic yeast genome. *Science*, *355*(6329), 1040–1044. https://doi.org/10.1126/science.aaf4557

Ross, M. J., & Coates, P. T. (2018). Using CRISPR to inactivate endogenous retroviruses in pigs: an important step toward safe xenotransplantation? *Kidney International*, *93*(1), 4–6. https://doi.org/10.1016/j.kint.2017.11.004

Schellenberger, J., Lewis, N. E., & Palsson, B. Ø. (2011). Elimination of thermodynamically infeasible loops in steady-state metabolic models. *Biophysical Journal*, *100*(3), 544–553. https://doi.org/10.1016/j.bpj.2010.12.3707

Seelig, G., Soloveichik, D., Zhang, D. Y., & Winfree, E. (2006). Enzyme-free nucleic acid logic circuits. *Science*, *314*(5805), 1585–1588. https://doi.org/10.1126/science.1132493

Sethuraman, N., & Stadheim, T. A. (2006). Challenges in therapeutic glycoprotein production. *Current Opinion in Biotechnology*, *17*(4), 341–346. https://doi.org/10.1016/j.copbio.2006.06.010

Shih, S. C. C., Goyal, G., Kim, P. W., Koutsoubelis, N., Keasling, J. D., Adams, P. D., Hillson, N. J., Singh, A. K. (2015). A versatile microfluidic device for automating synthetic biology. *ACS Synthetic Biology*, *4*(10), 1151–1164. https://doi.org/10.1021/acssynbio.5b00062

Sid, H., & Schusser, B. (2018). Applications of gene editing in chickens: A new era is on the horizon. *Frontiers in Genetics*, *9*, 456. https://doi.org/10.3389/fgene.2018.00456

Smith, H. O., Hutchison, C. A., Pfannkoch, C., & Venter, J. C. (2003). Generating a synthetic genome by whole genome assembly: phiX174 bacteriophage from synthetic oligonucleotides. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(26), 15440–15445. https://doi.org/10.1073/pnas.2237126100

Stark, J. C., Huang, A., Hsu, K. J., Dubner, R. S., Forbrook, J., Marshalla, S., Rodriguez F, Washington M, Rybnicky GA, Nguyen PQ, Hasselbacher B, Jabri R, Kamran R, Koralewski V, Wightkin W, Martinez T, Jewett, M. C. (2019). BioBits Health: Classroom Activities Exploring Engineering, Biology, and Human Health with Fluorescent Readouts. *ACS Synthetic Biology*, *8*(5), 1001–1009. https://doi.org/10.1021/acssynbio.8b00381

Stark, J. C., Huang, A., Nguyen, P. Q., Dubner, R. S., Hsu, K. J., Ferrante, T. C., Anderson M, Kanapskyte A, Mucha Q, Packett JS, Patel P, Patel R, Qaq D, Zondor T, Burke J, Martinez T, Miller-Berry A, Puppala A, Reichert K, Schmid M, Brand L, Hill LR, Chellaswamy JF, Faheem N, Fetherling S, Gong E, Gonzalzles EM, Granito T, Koritsaris J, Nguyen B, Ottman S, Palffy C, Patel A, Skweres S, Slaton A, Woods T, Donghia N, Pardee K, Collins JJ, Jewett, M. C. (2018). BioBits™ Bright: A fluorescent synthetic biology education kit. *Science Advances*, *4*(8), eaat5107. https://doi.org/10.1126/sciadv.aat5107

Stephens, N., Di Silvio, L., Dunsford, I., Ellis, M., Glencross, A., & Sexton, A. (2018). Bringing cultured meat to market: Technical, socio-political, and regulatory challenges in cellular agriculture. *Trends in Food Science & Technology*, *78*, 155–166. https://doi.org/10.1016/j.tifs.2018.04.010

Sundstrom, E. R., & Criddle, C. S. (2015). Optimization of Methanotrophic Growth and Production of Poly(3-Hydroxybutyrate) in a High-Throughput Microbioreactor System. *Applied and Environmental Microbiology*, *81*(14), 4767–4773. https://doi.org/10.1128/AEM.00025-15

Takahashi, M. K., Chappell, J., Hayes, C. A., Sun, Z. Z., Kim, J., Singhal, V., Spring KJ, Al-Khabouri S, Fall CP, Noireaux V, Murray RM, Lucks, J. B. (2015). Rapidly characterizing the fast dynamics of RNA genetic circuitry with cell-free transcription-translation (TX-TL) systems. *ACS Synthetic Biology*, *4*(5), 503–515. https://doi.org/10.1021/sb400206c

Tuerk, C., & Gold, L. (1990). Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. *Science*, *249*(4968), 505–510. https://doi.org/10.1126/science.2200121

Van Enennaam, A. (2018, June 12). Use of Gene Editing to Introduce the Polled Trait into Elite Germplasm. Retrieved February 26, 2019, from https://www.dairyherd.com/article/use-gene-editing-introduce-polled-trait-elite-germplasm/

Venayak, N., von Kamp, A., Klamt, S., & Mahadevan, R. (2018). MoVE identifies metabolic valves to switch between phenotypic states. *Nature Communications*, *9*(1), 5332. https://doi.org/10.1038/s41467-018-07719-4

Villa, J. K., Su, Y., Contreras, L. M., & Hammond, M. C. (2018). Synthetic biology of small rnas and riboswitches. *Microbiology Spectrum*, *6*(3). https://doi.org/10.1128/microbiolspec.RWR-0007-2017

Watkins, A. M., Geniesse, C., Kladwang, W., Zakrevsky, P., Jaeger, L., & Das, R. (2018). Blind prediction of noncanonical RNA structure at atomic accuracy. *Science Advances*, *4*(5), eaar5316. https://doi.org/10.1126/sciadv.aar5316

Weinhandl, K., Winkler, M., Glieder, A., & Camattari, A. (2014). Carbon source dependent promoters in yeasts. *Microbial Cell Factories*, *13*, 5. https://doi.org/10.1186/1475-2859-13-5

Wen, K. Y., Cameron, L., Chappell, J., Jensen, K., Bell, D. J., Kelwick, R., Kopniczky M, Davies JC, Filloux A, Freemont, P. S. (2017). A Cell-Free Biosensor for Detecting Quorum Sensing Molecules in P. aeruginosa-Infected Respiratory Samples. *ACS Synthetic Biology*, *6*(12), 2293–2301. https://doi.org/10.1021/acssynbio.7b00219

Wilkinson, M. D., Dumontier, M., Aalbersberg, I. J. J., Appleton, G., Axton, M., Baak, A.,

Blomberg N, Boiten JW, da Silva Santos LB, Bourne PE, Bouwman J, Brookes AJ, Clark T, Crosas M, Dillo I, Dumon O, Edmunds S, Evelo CT, Finkers R, Gonzalez-Beltran A, Gray AJ, Groth P, Goble C, Grethe JS, Heringa J, 't Hoen PA, Hooft R, Kuhn T, Kok R, Kok J, Lusher SJ, Martone ME, Mons A, Packer AL, Persson B, Rocca-Serra P, Roos M, van Schaik R, Sansone SA, Schultes E, Sengstag T, Slater T, Strawn G, Swertz MA, Thompson M, van der Lei J, van Mulligen E, Velterop J, Waagmeester A, Wittenburg P, Wolstencroft K, Zhao J, Mons, B. (2016). The FAIR Guiding Principles for scientific data management and stewardship. *Scientific Data*, 3, 160018. https://doi.org/10.1038/sdata.2016.18

Yang, K. K., Wu, Z., & Arnold, F. H. (2018). Machine learning-guided directed evolution for protein engineering. Retrieved from https://arxiv.org/abs/1811.10775v2

Yin, G., Garces, E. D., Yang, J., Zhang, J., Tran, C., Steiner, A. R., Roos C, Bajad S, Hudak S, Penta K, Zawada J, Pollitt S, Murray, C. J. (2012). Aglycosylated antibodies and antibody fragments produced in a scalable in vitro transcription-translation system. *MAbs*, *4*(2), 217–225. https://doi.org/10.4161/mabs.4.2.19202

You, M., & Jaffrey, S. R. (2015). Designing optogenetically controlled RNA for regulating biological systems. *Annals of the New York Academy of Sciences*, *1352*, 13–19. https://doi.org/10.1111/nyas.12660

Zhong, Z., & Liu, C. C. (2019). Probing pathways of adaptation with continuous evolution. *Current Opinion in Systems Biology*. https://doi.org/10.1016/j.coisb.2019.02.002