

Energy



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<u>Energy</u>

Energy focuses on the application of engineering biology tools and technologies to advance clean and affordable energy sources and to reduce overall energy consumption. To reduce the amount of carbon dioxide added to the atmosphere, energy will need to come from renewable sources, including waste gases (such as carbon dioxide and methane), microorganisms, and plants. Biology can be a source for renewable energy by providing biomass for electricity generation and for the production of highly energy-dense transportation fuels, and used to optimize processes to use less energy.

Societal Challenge 1: Produce affordable and clean energy.

- Science/Engineering Aim 1: Enable production of energy-dense and carbon-neutral transportation fuels from lignocellulosic feedstocks, oil crops, and agriculture and municipal wastes.
 - Engineering Biology Objective 1: Develop enzymes that can readily deconstruct lignin and cellulose/hemicellulose to monomers.
 - Gene Editing, Synthesis, and Assembly Achievement:
 - High-throughput synthesis of large gene clusters (> 10 kilobases).
 - Biomolecule, Pathway, and Circuit Engineering Achievement:
 - Engineer enzymes/pathways for production of hydrocarbons, including decarboxylating/decarbonylating enzymes, cofactor changes on enzymes, and new pathways/enzymes that conserve carbon/energy.
 - Engineer transporters to facilitate fuel export from the cell.
 - Improving enzymes for deconstruction of cellulosic biomass, including engineering cellulases and ligninases to be functional and stable in complex environments.
 - Host and Consortia Engineering Achievement:
 - Engineer microbes and/or consortia to efficiently express and secrete deconstruction enzymes.
 - Data Integration, Modeling, and Automation Achievement:
 - Technoeconomic and life cycle analysis models to determine sustainability of energy production.
 - Enzyme engineering models.
 - BioCAD models for designing gene expression.
 - Engineering Biology Objective 2: Further develop and advance oil crops that produce biofuels.
 - Gene Editing, Synthesis, and Assembly Achievement:
 - Edit chromosomes of oil crops to accumulate more and different types of oils.
 - Biomolecule, Pathway, and Circuit Engineering Achievement:
 - Engineer fatty acid synthases to produce fuels (fats) of a particular molecular weight.

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- Host and Consortia Engineering Achievement:
 - Engineer oil crops to be drought tolerant and not require significant inputs of fertilizer.
- Data Integration, Modeling, and Automation Achievement:
 - Accurate prediction of factors leading to increased yields for oil crops.
- Engineering Biology Objective 3: Develop crops suited to specific climates (particularly marginal lands that would not be used to grow food) that require little water or fertilizer and can be readily deconstructed to aromatic and sugar monomers.
 - Gene Editing, Synthesis, and Assembly Achievement:
 - Plant chromosome synthesis.
 - Synthesis of complex (e.g., repeat) DNA.
 - Efficient CRISPR systems for plants.
 - Methods for efficiently editing organelle genomes.
 - Biomolecule, Pathway, and Circuit Engineering Achievement:
 - Light energy conversion: engineer enzymes to more efficiently convert solar light to carbon/ATP.
 - Light capture: expand the range of solar spectrum wavelengths that can be captured by photosynthesis.
 - Improve CO₂ fixation by reducing 2-phosphoglycolate produced by RuBISCO O₂ fixation.
 - Host and Consortia Engineering Achievement:
 - Nitrogen fixation in plants.
 - Phosphate solubilization in plants.
 - Drought-tolerance traits in biomass crops.
 - Stable gene delivery to all plant tissues via viral vectors for prototyping of genetic designs.
 - Additional tools for controlling gene expression in plants, including large-scale knockout of unnecessary (for a given application or a particular environment) plant genes and pathways.
 - Metabolic pathways for producing bioproducts in plants (e.g., nonfuel products that could improve economics).
 - Methods to target and engineer specific microorganisms in plant microbiomes.
 - Co-regulation of plant and microbiome genes.
 - Develop microbiomes to aid nutrient uptake and water retention in soil.
 - Data Integration, Modeling, and Automation Achievement:
 - Models for identifying best geographic locations for energy crops.
 - Data collected at the plant- and field-level to understand growth and productivity.
 - Satellite imagery of plant productivity, land, and water use.



- Technoeconomic and life cycle analysis models to determine sustainability of energy production.
- BioCAD models for designing gene expression.
- Metabolic flux analysis of engineered organisms.
- Engineering Biology Objective 4: Develop microorganisms that can transform sugar and aromatic monomers into hydrocarbon-based liquid transportation fuels.
 - Gene Editing, Synthesis, and Assembly Achievement:
 - Synthesize large clusters of genes that encode metabolic pathways for various products (fuels, commodity chemicals, specialty chemicals, etc) ready to be transformed into any microbial host.
 - Biomolecule, Pathway, and Circuit Engineering Achievement:
 - Engineer transporters to facilitate fuel export from the cell.
 - Host and Consortia Engineering Achievement:
 - Develop efficient pathways for production of liquid transportation fuels from metabolic intermediates.
 - Develop metabolic pathways in microbes that will allow them to simultaneously consume aromatic monomers (from lignin) and sugars.
 - Data Integration, Modeling, and Automation Achievement:
 - Metabolic flux analysis of engineered organisms.
 - Models of microbes in bioreactors to predict performance.
 - Technoeconomic and life cycle analysis models to determine sustainability of energy production.
 - BioCAD models for designing gene expression.
- Science/Engineering Aim 2: Enable production of energy-dense and carbon-neutral transportation fuels (and other products) from C1 feedstocks (particularly carbon dioxide, carbon monoxide, and methane).
 - Engineering Biology Objective 1: Engineer microorganisms that can transform carbon dioxide and electron sources to liquid fuels.
 - Gene Editing, Synthesis, and Assembly Achievement:
 - Efficient CRISPR systems.
 - Methods for efficiently editing organelle genomes.
 - Biomolecule, Pathway, and Circuit Engineering Achievement:
 - Engineer enzymes/pathways for production of hydrocarbons, including decarboxylating/decarbonylating enzymes, cofactor changes on enzymes, and new pathways/enzymes that conserve carbon/energy.
 - Engineer transporters to facilitate fuel export from the cell.
 - Host and Consortia Engineering Achievement:
 - Develop efficient pathways for production of liquid transportation fuels from metabolic intermediates.



- Develop tools to enable engineering of carbon concentration/fixation pathways in CO₂ fixers.
- Large-scale knockout of unnecessary pathways.
- Development of carbon transport and concentration mechanisms.
- Organelle synthesis.
- Data Integration, Modeling, and Automation Achievement:
 - Metabolic flux analysis of engineered organisms.
 - Models of microbes in bioreactors to predict performance.
 - Technoeconomic and life cycle analysis models to determine sustainability of energy production.
 - BioCAD models for designing gene expression.
- Engineering Biology Objective 2: Engineer photosynthetic microorganisms that can transform sunlight and carbon dioxide to transportation fuels.
 - Gene Editing, Synthesis, and Assembly Achievement:
 - Efficient CRISPR systems.
 - Methods for efficiently editing organelle genomes.
 - Synthesize and transform genomes of organelles.
 - Biomolecule, Pathway, and Circuit Engineering Achievement:
 - Light energy conversion: engineer microbes and algae to more efficiently convert solar light to carbon/ATP.
 - Light capture: expand the range of solar spectrum wavelengths that can be captured by photosynthesis.
 - Improve CO₂ fixation by reducing 2-phosphoglycolate produced by RuBISCO O₂ fixation.
 - Engineer enzymes/pathways for production of hydrocarbons, including decarboxylating/decarbonylating enzymes, cofactor changes on enzymes, and new pathways/enzymes that conserve carbon/energy.
 - Engineer transporters to facilitate fuel export from the cell.
 - Host and Consortia Engineering Achievement:
 - Develop tools for controlling gene expression in photosynthetic microbes.
 - Develop efficient pathways for production of liquid transportation fuels from metabolic intermediates.
 - Development of carbon transport and concentration mechanisms.
 - Data Integration, Modeling, and Automation Achievement:
 - Technoeconomic and life cycle analysis models to determine sustainability of energy production.
 - BioCAD models for designing gene expression.
 - Metabolic flux analysis of engineered organisms.
 - Models of microbes in photobioreactors to predict performance.



- Engineering Biology Objective 3: Develop microorganisms that can transform methane to liquid transportation fuels.
 - Gene Editing, Synthesis, and Assembly Achievement:
 - Efficient CRISPR systems.
 - Synthesize large clusters of genes that encode metabolic pathways for various products (including fuels, commodity chemicals, and specialty chemicals) ready to be transformed into any microbial host.
 - Biomolecule, Pathway, and Circuit Engineering Achievement:
 - Recombinant expression of particulate and soluble methane monoxygenase (MMO) and demonstrate *in vivo* activity in a heterologous host.
 - Improve catalytic activity of MMO in native and heterologous hosts.
 - Engineer enzymes/pathways for production of hydrocarbons, including decarboxylating/decarbonylating enzymes, cofactor changes on enzymes, and new pathways/enzymes that conserve carbon/energy.
 - Engineer transporters to facilitate fuel export from the cell.
 - Host and Consortia Engineering Achievement:
 - Growth on alternative carbon sources to allow systematic genetic screening of critical enzymes for methane utilization.
 - More efficient and rapid transformation methods.
 - Large-scale knockout of unnecessary pathways.
 - Develop efficient pathways for production of liquid transportation fuels from metabolic intermediates.
 - Data Integration, Modeling, and Automation Achievement:
 - Metabolic flux analysis of engineered organisms.
 - Models of microbes in bioreactors to predict performance.
 - Technoeconomic and life cycle analysis models to determine sustainability of energy production.
 - BioCAD models for designing gene expression.
- Engineering Biology Objective 4: Engineer microorganisms that can transform carbon dioxide and electron sources to methane.
 - Gene Editing, Synthesis, and Assembly Achievement:
 - Efficient CRISPR systems.
 - Methods for efficiently editing organelle genomes.
 - Synthesize and transform genomes of organelles.
 - Biomolecule, Pathway, and Circuit Engineering Achievement:
 - Light energy conversion: engineer microbes and algae to more efficiently convert solar light to carbon/ATP.
 - Light capture: expand the range of solar spectrum wavelengths that can be captured by photosynthesis.



- Improve CO₂ fixation by reducing 2-phosphoglycolate produced by RuBISCO O₂ fixation.
- Engineer enzymes/pathways for production of hydrocarbons, including decarboxylating/decarbonylating enzymes, cofactor changes on enzymes, and new pathways/enzymes that conserve carbon/energy.
- Engineer transporters to facilitate fuel export from the cell.
- Engineer enzymes/pathways for production of hydrogen (via water splitting).
- Host and Consortia Engineering Achievement:
 - Develop tools for controlling gene expression in photosynthetic microbes.
 - Develop efficient pathways for production of liquid transportation fuels from metabolic intermediates.
 - Development of carbon transport and concentration mechanisms.
- Data Integration, Modeling, and Automation Achievement:
 - Technoeconomic and life cycle analysis models to determine sustainability of energy production.
 - BioCAD models for designing gene expression.
 - Metabolic flux analysis of engineered organisms.
 - Models of microbes in photobioreactors to predict performance.
- Science/Engineering Aim 3: Enable the efficient production of biomass for conversion to electricity.
 - Engineering Biology Objective 1: Develop crops that require little water or fertilizer and can be used to generate biomass designed for eventual electricity production.
 - Gene Editing, Synthesis, and Assembly Achievement:
 - Plant chromosome synthesis.
 - Synthesis of complex (e.g., repeat) DNA.
 - Biomolecule, Pathway, and Circuit Engineering Achievement:
 - Engineer proteins that improve the water acquisition in plant (aquaporins).
 - Engineer proteins that reduce water loss (uniport aquaporins).
 - Host and Consortia Engineering Achievement:
 - Improve plant transformation protocols.
 - Reduce generation time of model plant species.
 - Data Integration, Modeling, and Automation Achievement:
 - Risk assessment models for the impact of engineered plants on the surrounding ecology.
 - Methods to capture field data (such as growth, microbe composition) under non-standardized conditions over a long period of time.



- Engineering Biology Objective 2: Engineer organisms (including plants, microorganisms, and algae) to more efficiently convert solar light to fix carbon and produce ATP.
 - Gene Editing, Synthesis, and Assembly Achievement:
 - Plant chromosome synthesis.
 - Synthesis of complex (e.g., repeat) DNA.
 - Biomolecule, Pathway, and Circuit Engineering Achievement:
 - Improve efficiency of extracellular electron transfer from easy-togrow microbes (e.g., *Shewanella*).
 - Light energy conversion: engineer microbes and algae to more efficiently convert solar light to carbon/ATP.
 - Light capture: expand the range of solar spectrum wavelengths that can be captured by photosynthesis.
 - Improve CO₂ fixation by reducing 2-phosphoglycolate produced by RuBISCO O₂ fixation.
 - Engineer plants with a higher content of lignin and lower cellulose/hemicellulose content to enable greater biomass production.
 - Host and Consortia Engineering Achievement:
 - Convert extracellular electron transfer of diverse redox potential metals from *Geobacter* species to *Shewanella* species.
 - Develop fast-growing, drought-tolerant grasses and trees for specific environments.
 - Data Integration, Modeling, and Automation Achievement:
 - Risk assessment model for the impacts of engineered plants on the surrounding ecology.
 - Methods to capture field data (such as growth, microbe composition) under non-standardized conditions over a long period of time.

Societal Challenge 2: Reduce global energy consumption.

• Science/Engineering Aim 1: Further develop energy-saving processes with biology.

- Engineering Biology Objective 1: Develop and advance more efficient enzymes for everyday use (such as for laundry and dishwasher detergent).
 - Gene Editing, Synthesis, and Assembly Achievement:
 - Efficient CRISPR systems for more organisms.
 - Biomolecule, Pathway, and Circuit Engineering Achievement:
 - Improve proteases, cellulases, lipases, and amylases to enable/improve activity at low temperature, stability during storage, stability in aqueous detergent and activity in the presence of other enzymes.
 - Host and Consortia Engineering Achievement:
 - Efficient protein production and purification.



- Data Integration, Modeling, and Automation Achievement:
 - Computational enzyme design.
 - Enzyme engineering models.
 - BioCAD models for designing gene expression.
- Science/Engineering Aim 2: Produce more energy-efficient crops that require less energy input for cultivation.
 - Engineering Biology Objective 1: Develop crops that require little water or fertilizer to reduce energy used for cultivation.
 - Gene Editing, Synthesis, and Assembly Achievement:
 - Efficient CRISPR systems for plants.
 - Biomolecule, Pathway, and Circuit Engineering Achievement:
 - Drought-toleration synthetic circuits that close pores in response to environmental or externally-delivered signals.
 - Host and Consortia Engineering Achievement:
 - Produce model plants that have a higher yield.
 - Efficient plant transformation technologies (including for chloroplast and genome) for energy relevant plants.
 - Data Integration, Modeling, and Automation Achievement:
 - Risk assessment models for the impact of engineered plants on the surrounding ecology.
 - Methods to capture field data (such as growth, microbial composition) under non-standardized conditions over a long period of time.
 - Engineering Biology Objective 2: Expand the range of solar spectrum wavelengths that can be captured by photosynthesis.
 - Gene Editing, Synthesis, and Assembly Achievement:
 - Efficient CRISPR systems for plants.
 - Biomolecule, Pathway, and Circuit Engineering Achievement:
 - Rational engineering and directed evolution of the photosynthetic light-harvesting complex.
 - Host and Consortia Engineering Achievement:
 - Efficient expression of functional designed enzymes.
 - Data Integration, Modeling, and Automation Achievement:
 - Modeling of photosynthesis and use of model to design a photosynthetic apparatus that captures more light.
- Science/Engineering Aim 3: Develop organismal bio-processes that enable the production of energy from (currently) atypical sources.
 - Engineering Biology Objective 1: Enable seawater fermentation.
 - Gene Editing, Synthesis, and Assembly Achievement:
 - Develop tools/methods for editing the chromosomes of microbes resident in seawater.
 - Biomolecule, Pathway, and Circuit Engineering Achievement:
 - Biosynthetic pathways active in halophilic/hypersaline conditions.

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- Host and Consortia Engineering Achievement:
 - Develop robust genome engineering for halophiles (e.g., *Vibrio natriegens*).
 - Engineer resistance to phage/toxins.
 - Strict biocontainment strategies for potential engineered organisms deployment.
- Data Integration, Modeling, and Automation Achievement:
 - Modeling of organism fitness in seawater.
- Engineering Biology Objective 2: Enable wastewater fermentation.
 - Gene Editing, Synthesis, and Assembly Achievement:
 - Develop tools/methods for editing the chromosomes of microbes resident in wastewater treatment facilities.
 - Biomolecule, Pathway, and Circuit Engineering Achievement:
 - Engineer enzymes to convert fats in wastewater to hydrocarbons.
 - Host and Consortia Engineering Achievement:
 - Engineer resistance to phage/toxins.
 - Strict biocontainment strategies for potential engineered organisms deployment.
 - Engineer microbes for ammonium oxidation, denitrification, and polyphosphate accumulation.
 - Data Integration, Modeling, and Automation Achievement:
 - Model competition of microbes in wastewater treatment facilities to predict survival of engineered organisms.



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