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Kristala Jones Prather's Lecture Part 1: Introduction to Synthetic Biology and Metabolic Engineering

Teaching Tools were prepared by Tess Veuthey.

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Synthetic biology, applied engineering, metabolic engineering, biological photography, nitrogen fixing, biofuels

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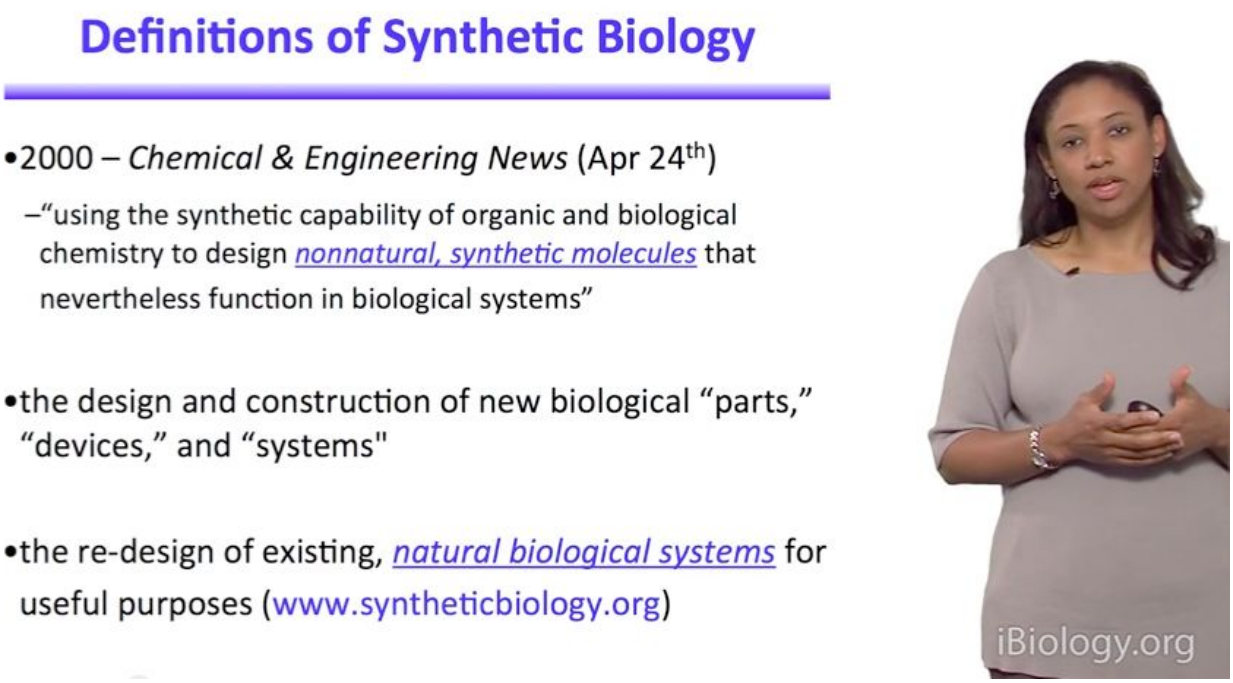
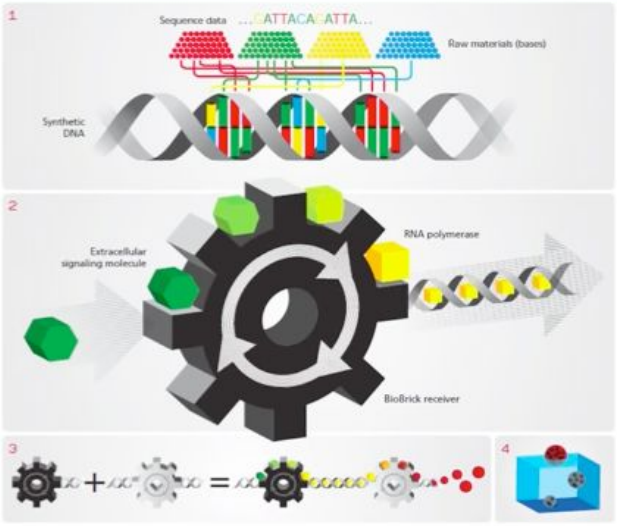


[3:08]
The definition of Synthetic Biology has expanded from (1) using biology to create nonnatural molecules to also include (2) re-purposing and re-designing natural molecules and pathways for useful purposes.

Building Biological Machines



http://seedmagazine.com/content/article/cribsheet_16_synthetic_biology/

Practical Definition of Synthetic Biology

- "making biology easier to engineer" -- Synthetic Biology Engineering Research Center (SynBERC)
- Applying engineering principles to biological systems
  - –Design
  - –Modeling
  - –Characterization
  - –Abstraction

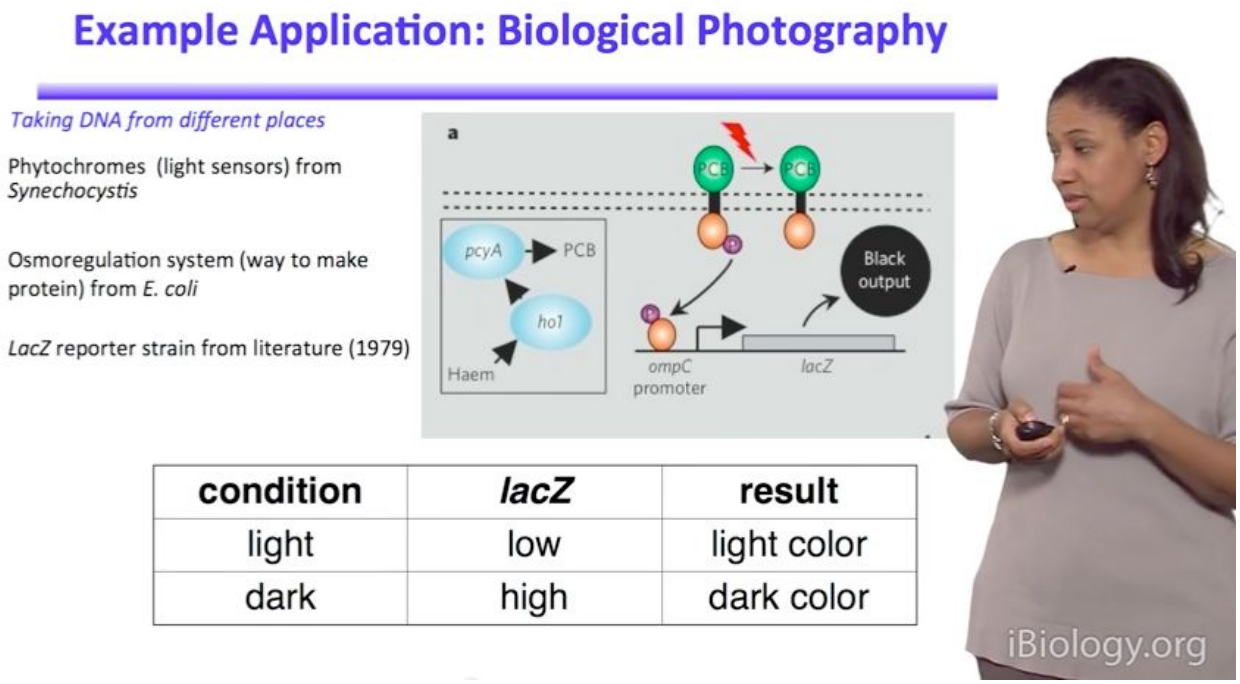
Key enabling technology:

DNA synthesis

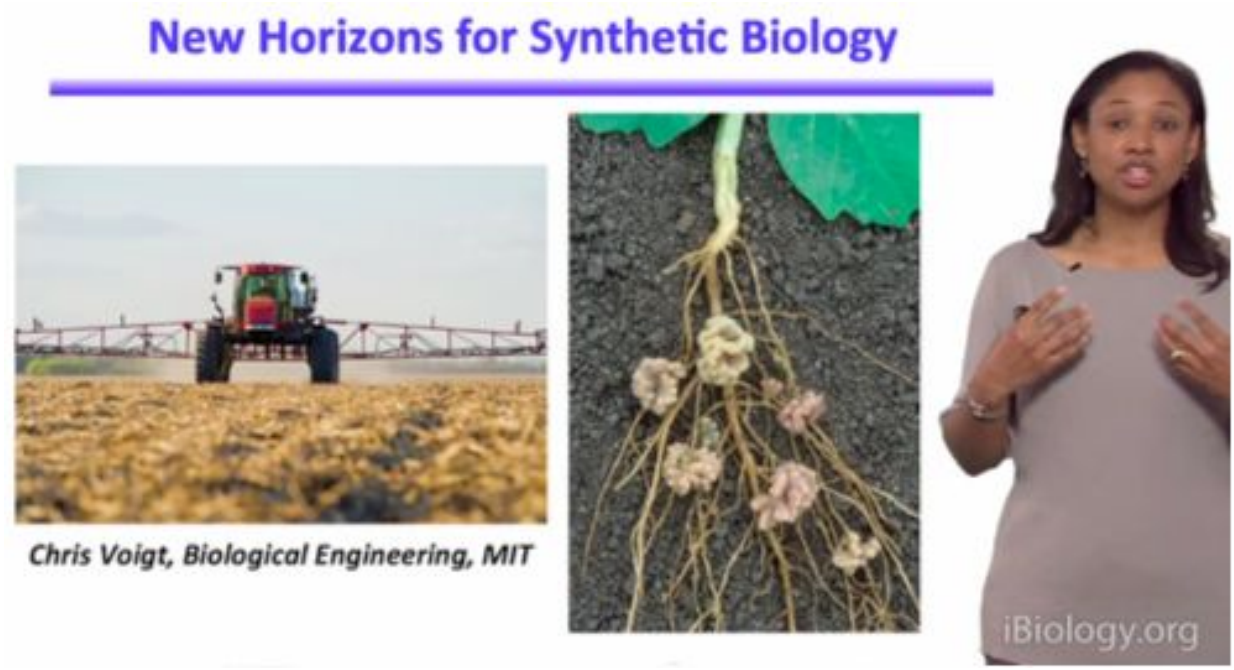
[5:14] and [6:58]

Synthetic Biology applies engineering principles to biological systems, and specifically uses DNA as the encoding molecule for new biological pathways. Key principles include design (building with a specific function in mind), modeling (writing equations that show understanding of underlying principles and support the design), characterization (constructing and testing of both modular components and whole systems), and

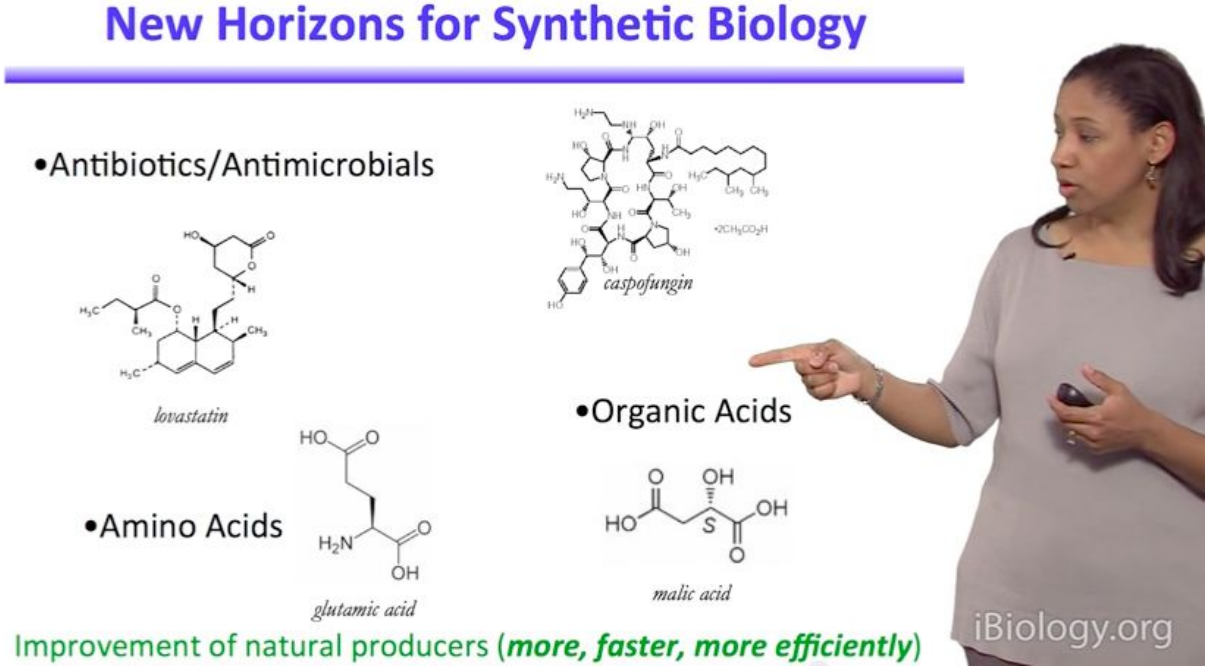
abstraction (stepping back and generalizing lessons learned in one context to the design of other systems, including systems that string together modules from previously separate pathways).



[10:03]
In this example of synthetic biology, a biological photography system is designed by bringing together pieces from different pathways. These include (1) a light sensor, (2) a way to trigger protein expression, and (3) a protein that causes cell pigmentation.

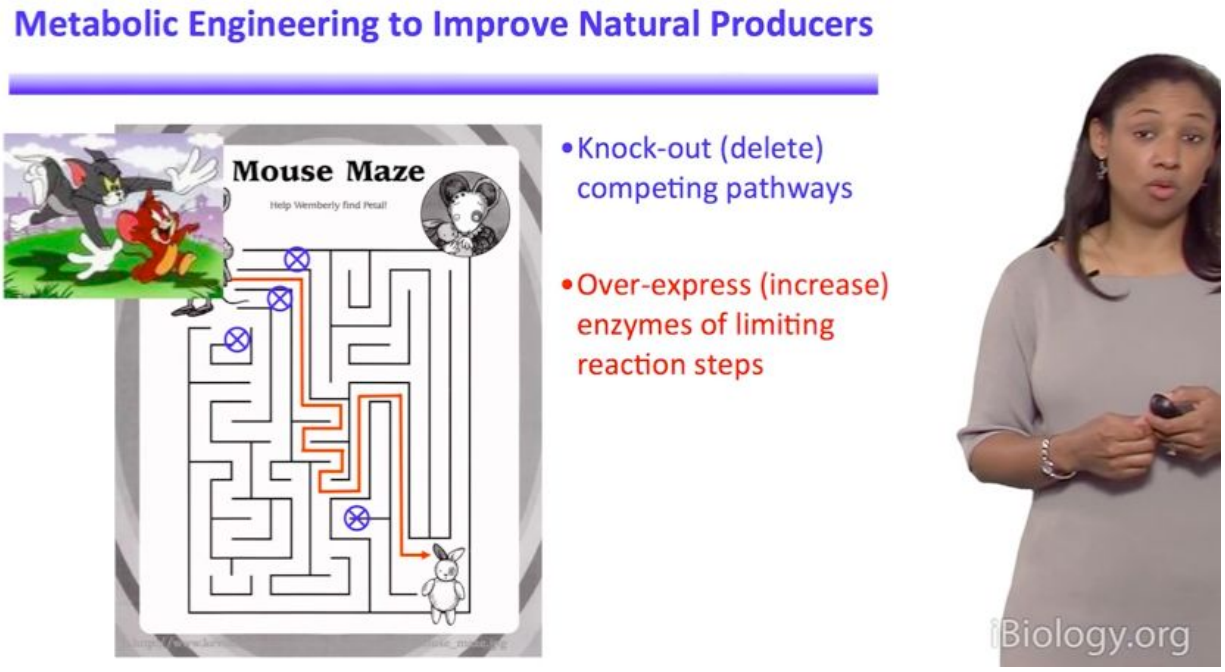


[13:42]
Engineering is a practical, problem-based field, and many synthetic biology groups are focused on solving challenging real-world problems. These include engineering pancreatic stem cells to replenish those lost in Type 1 Diabetes, and giving plants nitrogen-fixing abilities to avoid the use of toxic fertilizers.



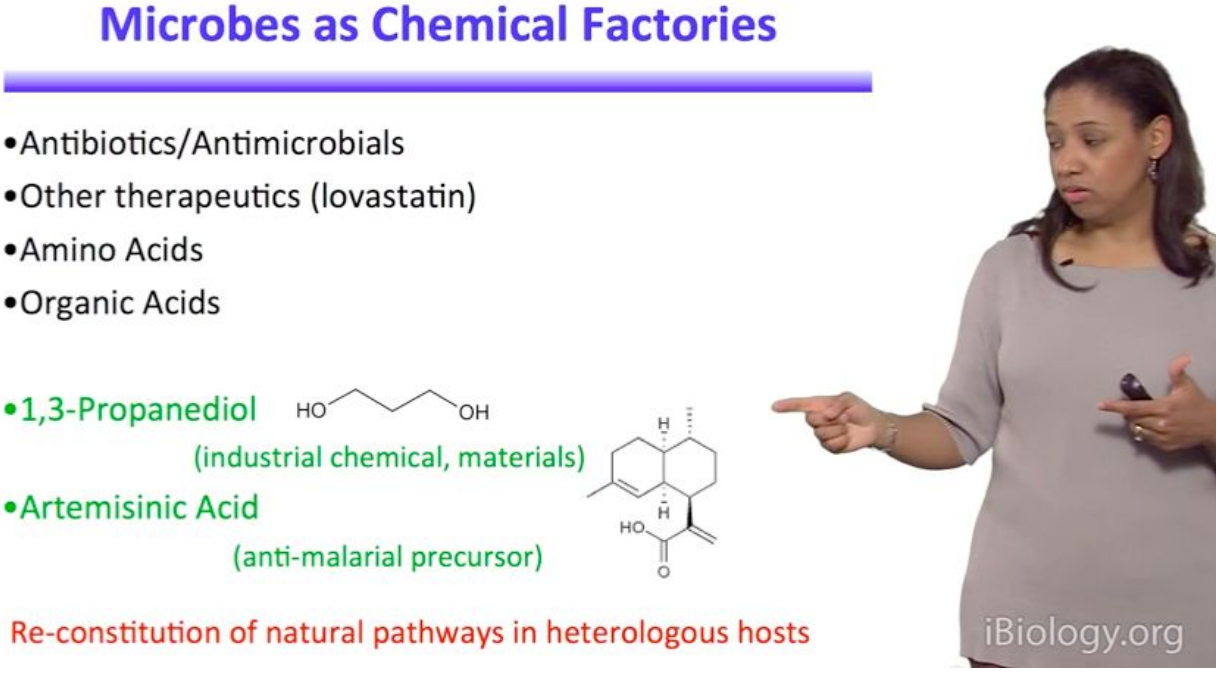
[18:10]

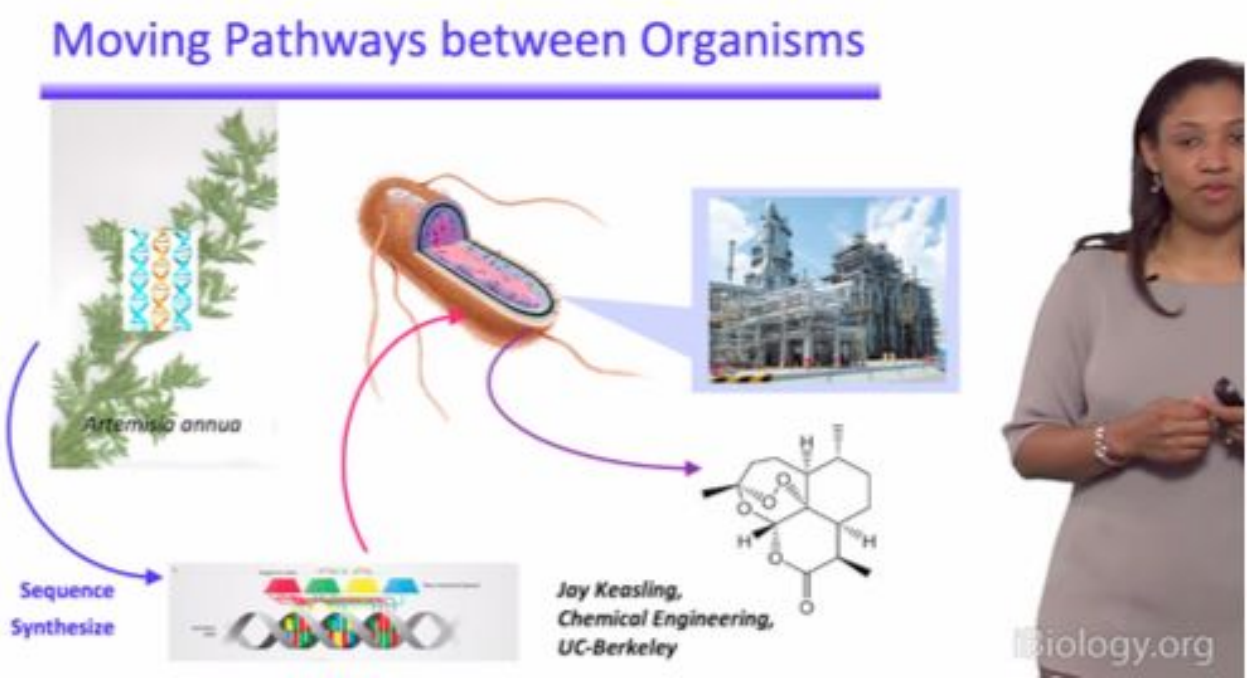
Metabolic engineering is focused on biology's ability to do chemistry, particularly to create useful complex molecules like antibiotics. Researchers in synthetic biology are trying to improve biology's ability to make these molecules, specifically focusing on production quantity, speed, and yield.



[19:49]

Using a maze analogy, we can explain how metabolic engineering can facilitate desired biological products within an organism. We can knockout dead-end routes (processes that lead to byproducts), and add 'motivating factors' that speed production along the desired path (modifying the rate-limiting steps that normally slow down the desired pathway)

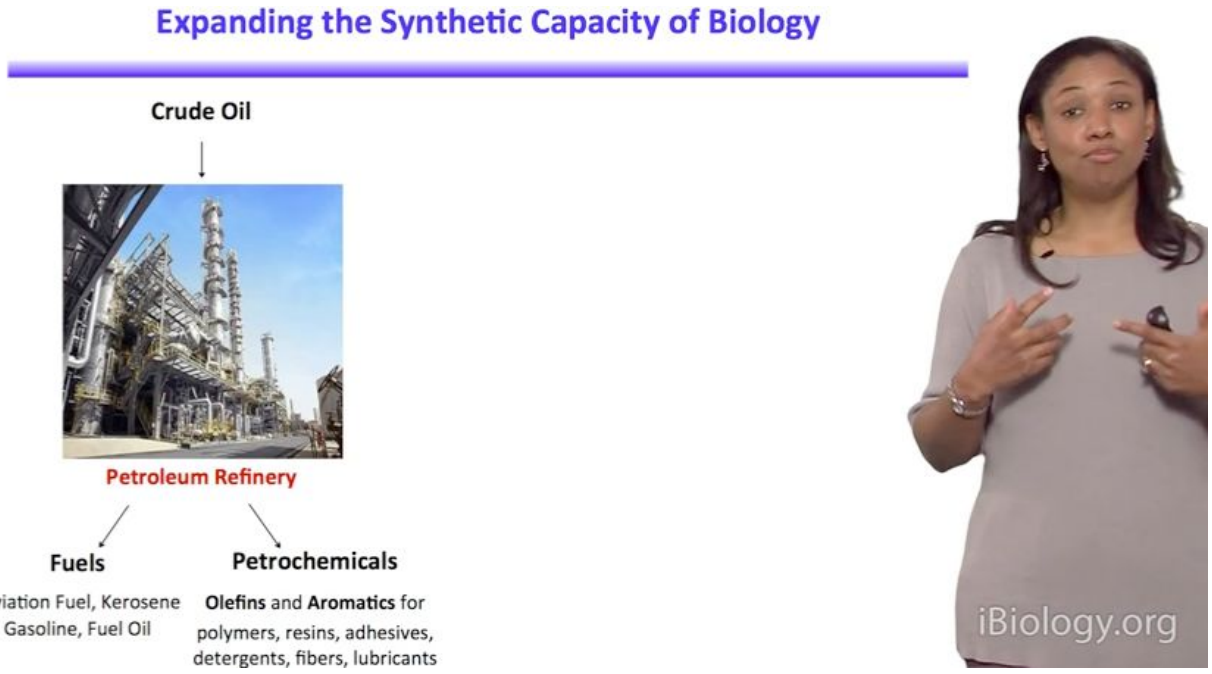


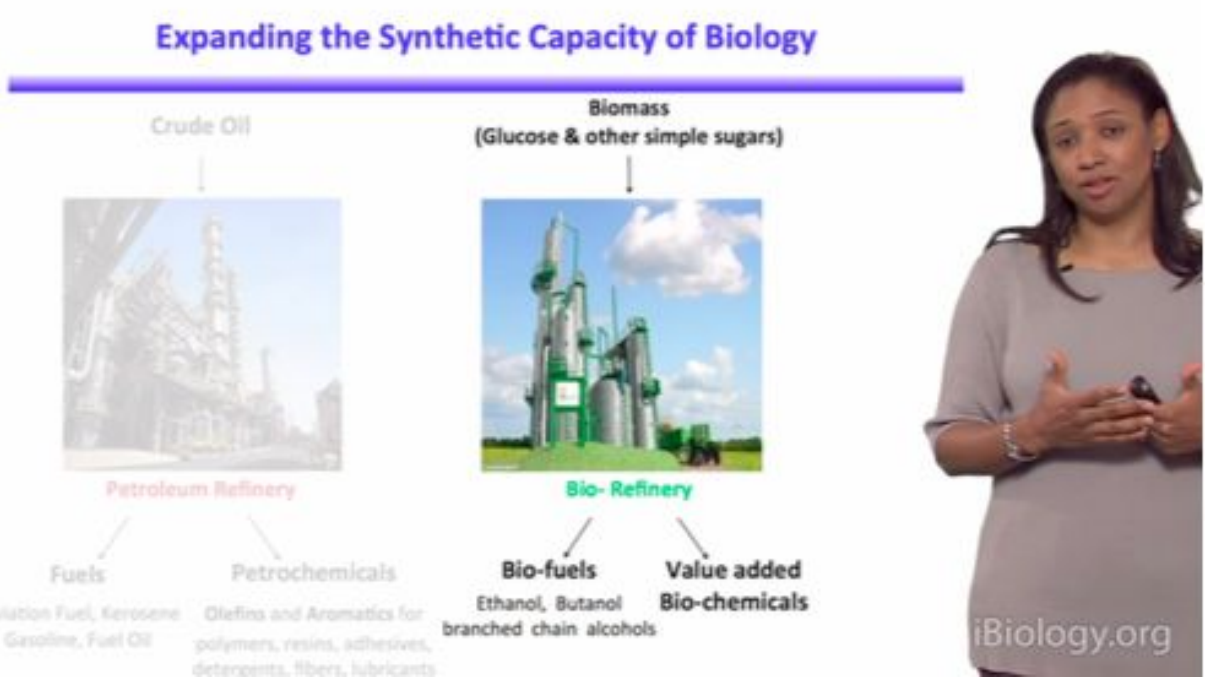


[20:52] and [22:25]

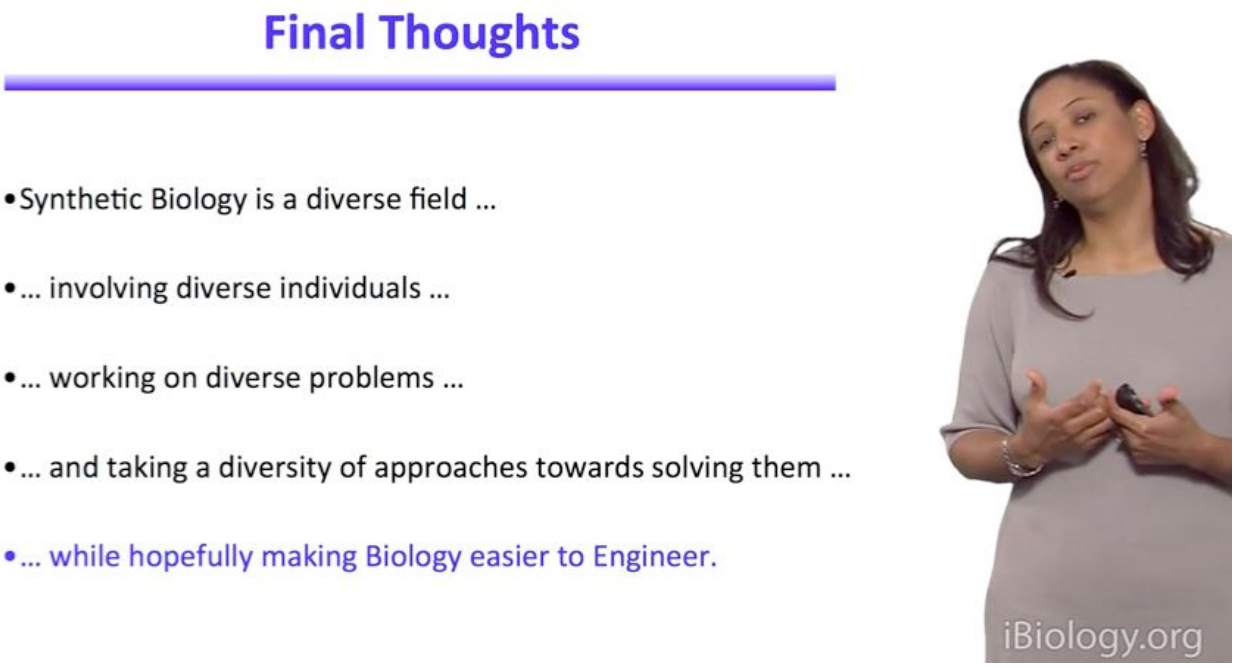
Synthetic biology is used to speed up production of molecules made by a single organism (first four examples), but it can also combine production modules from different organisms into a single producer. In this case, each step of the process does not 'match' its original context and may interact with steps originating from other organisms. Researchers do a lot of optimization to get the process to work as a whole.

One example of this is the production of artemisinic acid, which is normally made in a plant, but can be made at larger scales in yeast. The appropriate DNA needs to be identified and extracted from the plant and then expressed in the yeast.





[23:13] and [23:38]
Prof. Prather's lab is expanding biology's ability to do chemistry, particularly tackling production of fuel and petrochemical substitutes. While fuel molecules are used for their energy properties, petrochemicals are used for their mass properties and made into lubricants, adhesives, and other useful substances.
Prof. Prather's lab is trying to recreate these useful mass properties using biomass as a starting molecule instead of crude oil. Biomass would provide starting chemicals like glucose and sugars, which living things use for energy and as starting points for useful molecules.



[25:36]
While all of synthetic biology uses DNA as the central encoding molecule, the field is otherwise very diverse. Scientists and engineers with different perspectives take many different approaches to create useful molecules for a wide range of applications, including health, energy, and environmental management.

3. Review Questions

1. What is a modern definition of synthetic biology?

a. Using bacteria to produce non-natural, synthetic molecules.
b. Re-design and re-purposing of natural biological systems for useful purposes.
c. Using industrial chemical methods to create useful biological compounds.

2. What is the hallmark molecule in synthetic biology?

    a. RNA
    b. Proteins
    c. Transcription Factors
    d. Phosphates
    e. DNA

3. How does biological photography work?

    a. Exposure to chemicals can bleach dark bacteria to create a pattern.
    b. Light causes a surface protein to change conformation and become a different color.
    c. Light causes a transcription factor to be expressed, leading to the expression of a dark reporter molecule.

4. A ‘heterologous host’ is a:

    a. Production host in which a single native pathway is optimized.
    b. Production host in which many pathways with different products are optimized.
    c. Production host in which pathways from other organisms are inserted and expressed.
    d. A recipient organism that optimally stores excess product from other organisms.

5. What is Prof. Prather’s lab currently working on?

    a. Engineering bacteria to make gasoline.
    b. Engineering yeast to consume crude oil released in oil spills.
    c. Engineering bacteria to produce bio-fuels.
    d. Engineering microbes to produce bio-chemicals to replace Petrochemicals.

4. Answers to Review Questions

1. B

2. E
3. C
4. C
5. D

5. Discussion Questions

1. What are some key differences between science and engineering? How does synthetic biology bridge these fields?
2. What principles of engineering are used in synthetic biology?
    a. Map these onto another engineering process.
    b. How are these principles applied in other areas of biology research?
3. What are some real-world applications of synthetic biology, particularly using hosts other than yeast and bacteria?
4. What are additional challenges to implementing synthetic biology outside of the lab?

6. Answers to Discussion Questions

1. Science is primarily concerned with discovering how the natural world works. Engineering is focused on inventing practical solutions to real-world problems. Engineers' first concern is that something works at all. A process can be optimized empirically, even when the mechanisms of some of the components are not fully understood. Synthetic biology uses DNA, genes, and biological processes as 'off-the-shelf' modular components that can be thought of outside of their host organisms. While synthetic biologists study and manipulate cellular processes, the goal of most studies is simply to produce a desired output.
2. Many engineering principles are used in synthetic biology, but Prof. Prather focuses on design, modeling, characterization, and abstraction.

a. Map these onto another engineering process.

Design	Modeling	Characterization	Abstraction
Designing a commercial object (eg: phone case)	Producing both virtual (CAD) and physical mock-ups to confirm the assumptions made in the design. (eg: Does the phone slide into the case properly? Is it possible to manufacture?)	Testing the object's ability to fulfill its function and observing any unpredicted issues. (eg: How well does the case protect the phone from falls and other accidents? How easily does the case break? Does it have good grip?)	Learning from the Design → modeling → characterization process. (eg: Does this new plastic have material properties that could be useful for something else? Does a solid vs. grid-like plastic configuration maintain protection while decreasing cost?)

b. These principles are applied to the design of experiments, which are in turn used for science discoveries.

Design	Modeling	Characterization	Abstraction
What is the goal of this experiment? We will test whether protein X is required for rostral-caudal limb patterning.	How does this claim fit into our established framework and knowledge base? Where is X found? During what time period of development is it expressed? Can we make a knock-out model (KO)?	Does our KO produce the expected phenotype? Is it a complete KO? What do the heterozygous animals look like? If we do live-imaging of protein X, does it do anything unexpected? Is it cleaved? Is it trafficked around the cell?	Did these experiments teach us about how to study protein X and other developmentally important proteins? Are KO the most robust way to see a phenotype? Do fluorescent dominant-negative mutations give us important information on protein behavior?

3. Ron Weiss's lab is working on stem-cells that would (1) automatically detect the amount of insulin-producing pancreatic stem cells (Beta cells) available in the body and (2) differentiate into Beta cells if the population level was too low. Beta cells are destroyed in Type I Diabetes, but, with this mechanism, an engineered pool of Beta cells could be continually replenished to the necessary level.

   Chris Voigt's lab is trying to give plants nitrogen-fixing abilities to avoid their dependence on nitrogen-fixing bacteria and fertilizers. Because plants are sensitive to growing conditions that are hard to control at a large scale (temperature, light, water, etc), they are not good hosts for the production of chemicals that need to be extracted. However, plants themselves are important products, so using synthetic biology to help them grow in a larger variety of conditions is very useful.

4. While labs can do proof-of-principle production of molecules using synthetic biology, eventually many of these products would need to be mass-produced to be useful. Large-scale production will lead to a host of new issues, analogous to issues that arose during mass-production of objects during the industrial revolution. Concerning biofuels and value-added biochemicals, where will the biomass come from? What will the ideal host be? What will be the byproducts of each production process? Will there be constraining needs on water, land, and waste treatment? Would synthetically engineered microbes be able to survive outside the factory environment? If so, could they colonize humans? Do they pose toxicity risks? If the artificial DNA mutates, could large amounts of mutated bacteria produce harmful substances?

7. Questions for Discussion Paper

Sheppard, M. J., Kunjapur, A. M., Wenck, S. J. & Prather, K. L. J. Retro-biosynthetic screening of a modular pathway design achieves selective route for microbial synthesis of 4-methyl-pentanol. Nat Commun 5, (2014).

1. Describe one synthetic biology application that would be useful for your personal life and another application that would be useful for your research
2. Summarize this paper using the 5-sentence grant-proposal structure outlined below:

a. What is the problem?
b. What are the knowledge gaps that limit current solutions?
c. What is the specific insight/technology used that will overcome this?
d. How do they solve the problem using this technology?
e. What is the next problem?

3. Many aspects of this artificial process need to be checked and optimized. Pick one of these processes (listed below) and explain the experiments involved.

    a. Substrate specificity (Fig. 2,3)
    b. Avoidance of futile cycles (Fig 3)
    c. Reducing products from shunts (Fig. 3)
    d. Efficiency (Fig. 4)
    e. Operon order (pg. 5).

4. How can this process be further optimized or iterated upon?

8. Answers to Questions for Discussion Paper

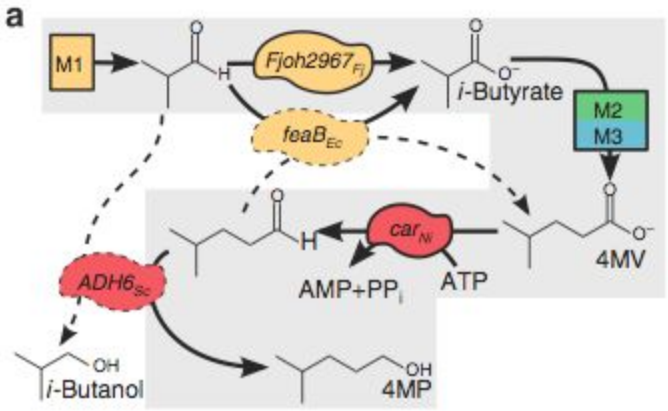
1. This is an open question for students to see what they come up with!

2. Summarize this paper using the 5-sentence grant-proposal structure outlined below:

    a. What is the problem? Fossil fuels are not sustainable and there are no viable biofuel replacements.
    b. What are the knowledge gaps that limit current solutions? The current technology uses alpha-ketoacid elongation, which causes a redox imbalance that limits the maximum pathway efficiency.
    c. What is the specific insight/technology used that will overcome this? New synthesis pathways can be put together by combining enzymes from different organisms.
    d. How do they solve the problem using this technology? 4-methyl-pentanol (4MP) was created by reverse-engineering a production system using components from different microbes.
    e. What is the next problem? How can we further optimize this process to make it more effective (more product) in addition to more efficient (more yield per substrate)? Can this product be made in anaerobic conditions? Is it possible to increase production to an industrial scale?

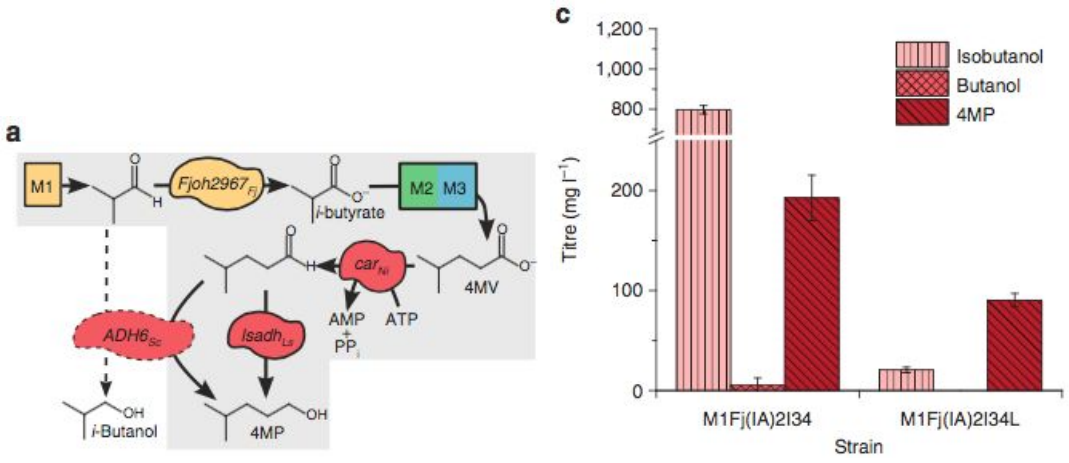
3. Many aspects of this artificial process need to be checked and optimized. Pick one of these processes (listed below) and explain the experiments involved.

    a. Substrate specificity (Fig. 2,3) The carboxylic acid reductase Car_{Ni} was selected from a set of Car enzymes because it has a preference for longer carbon chains, particularly 4MV and 4-methyl-hexanoate. It has a low activity on isobutyrate and butyrate, which are precursors in the desired pathway. $FeaB_{EC}$ could accept multiple substrates, including a downstream product, leading to a futile cycle (see part b). Swapping out another enzyme with higher substrate specificity eliminated the futile cycle and improved yield.
    b. Avoidance of futile cycles (Fig 3) Since 4MV (an intermediate product) was present even when the full process was assembled, the researchers hypothesized that $FeaB_{EC}$ was oxidizing 4-methyl-valeraldehyde to 4MV. FeaBEC was replaced with $Fjoh2967_{Fj}$, which eliminated the futile cycle and improved efficiency.



    c. Efficiency (Fig. 4)

        In module 4, $ADH6_{SC}$ produced large amount of the byproduct isobutanol. Replacing $ADH6_{SC}$ with $Lsadh_{LS}$ reduced the proportion of isobutanol byproduct created but also decreased the absolute amount of 4MP. While this swap improved the efficiency of the process, it reduced the efficacy.



4. This is also an open ended question for students.