
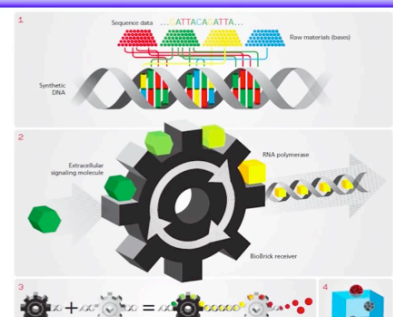




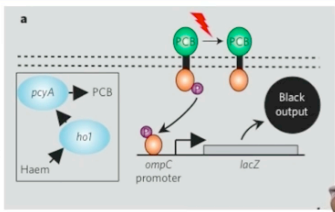

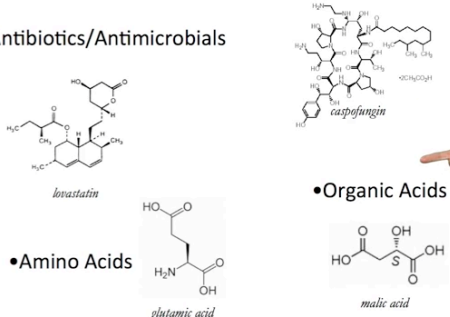
**Lecture 1: Introduction to Synthetic Biology and Metabolic Engineering**



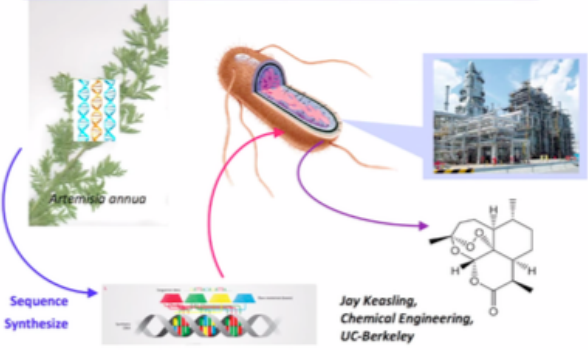


**Key words and terms**



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

**Lecture notes**

<p><b>Definitions of Synthetic Biology</b></p> <ul style="list-style-type: none"> <li>•2000 – <i>Chemical &amp; Engineering News</i> (Apr 24<sup>th</sup>)              –“using the synthetic capability of organic and biological chemistry to design <u>nonnatural, synthetic molecules</u> that nevertheless function in biological systems”</li> <li>•the design and construction of new biological “parts,” “devices,” and “systems”</li> <li>•the re-design of existing, <u>natural biological systems</u> for useful purposes (<a href="http://www.syntheticbiology.org">www.syntheticbiology.org</a>)</li> </ul> 	<p>[3:08] The definition of Synthetic Biology has expanded from (1) using biology to create non-natural molecules to also include (2) re-purposing and re-designing natural molecules and pathways for useful purposes.</p>
<p><b>Building Biological Machines</b></p>  <p><a href="http://seedmagazine.com/content/article/cribsheet_16_synthetic_biology/">http://seedmagazine.com/content/article/cribsheet_16_synthetic_biology/</a></p> 	<p>[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</p>
<p><b>Practical Definition of Synthetic Biology</b></p> <ul style="list-style-type: none"> <li>•“making biology easier to engineer” -- Synthetic Biology Engineering Research Center (SynBERC)</li> <li>•Applying engineering principles to biological systems             <ul style="list-style-type: none"> <li>–Design</li> <li>–Modeling</li> <li>–Characterization</li> <li>–Abstraction</li> </ul> </li> </ul> <div style="border: 1px solid black; padding: 5px; display: inline-block;"> <p>Key enabling technology: DNA synthesis</p> </div> 	<p>(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</p>

	<p>that string together modules from previously separate pathways).</p>									
<p><b>Example Application: Biological Photography</b></p> <p>Taking DNA from different places</p> <p>Phytochromes (light sensors) from <i>Synechocystis</i></p> <p>Osmoregulation system (way to make protein) from <i>E. coli</i></p> <p><i>lacZ</i> reporter strain from literature (1979)</p>  <table border="1" data-bbox="341 651 812 756"> <thead> <tr> <th>condition</th> <th><i>lacZ</i></th> <th>result</th> </tr> </thead> <tbody> <tr> <td>light</td> <td>low</td> <td>light color</td> </tr> <tr> <td>dark</td> <td>high</td> <td>dark color</td> </tr> </tbody> </table> <p>iBiology.org</p>	condition	<i>lacZ</i>	result	light	low	light color	dark	high	dark color	<p>[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.</p>
condition	<i>lacZ</i>	result								
light	low	light color								
dark	high	dark color								
<p><b>New Horizons for Synthetic Biology</b></p>  <p>Chris Voigt, Biological Engineering, MIT</p> <p>iBiology.org</p>	<p>[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.</p>									
<p><b>New Horizons for Synthetic Biology</b></p> <ul style="list-style-type: none"> <li>•Antibiotics/Antimicrobials</li> <li>•Amino Acids</li> </ul>  <p>Improvement of natural producers (<b>more, faster, more efficiently</b>)</p> <p>iBiology.org</p>	<p>[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.</p>									

<p><b>Metabolic Engineering to Improve Natural Producers</b></p>  <ul style="list-style-type: none"> <li>• Knock-out (delete) competing pathways</li> <li>• Over-express (increase) enzymes of limiting reaction steps</li> </ul> 	<p>[19:49] Using a maze analogy, we can explain how metabolic engineering can facilitate desired biological products within an organism. We can knock-out dead-end routes (processes that lead to by-products), and add 'motivating factors' that speed production along the desired path (modifying the rate-limiting steps that normally slow down the desired pathway)</p>
<p><b>Microbes as Chemical Factories</b></p> <ul style="list-style-type: none"> <li>• Antibiotics/Antimicrobials</li> <li>• Other therapeutics (lovastatin)</li> <li>• Amino Acids</li> <li>• Organic Acids</li> </ul> <p>• 1,3-Propanediol <chem>OCC(O)CO</chem>          (industrial chemical, materials)</p> <p>• Artemisinic Acid <chem>CC1=C(C)C(C)C(C)C1C(=O)O</chem>          (anti-malarial precursor)</p> <p>Re-constitution of natural pathways in heterologous hosts</p> <p><b>Moving Pathways between Organisms</b></p>   	<p>[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</p>

<p style="text-align: center;"><b>Expanding the Synthetic Capacity of Biology</b></p> <hr style="border: 1px solid purple;"/> <div style="display: flex; justify-content: space-around;"> <div style="text-align: center;"> <p>Crude Oil</p>  <p><b>Petroleum Refinery</b></p> <div style="display: flex; justify-content: space-between;"> <div style="text-align: left;"> <p><b>Fuels</b></p> <p>Aviation Fuel, Kerosene Gasoline, Fuel Oil</p> </div> <div style="text-align: left;"> <p><b>Petrochemicals</b></p> <p>Olefins and Aromatics for polymers, resins, adhesives, detergents, fibers, lubricants</p> </div> </div> </div> <div style="text-align: center;"> <p>Biomass (Glucose &amp; other simple sugars)</p>  <p><b>Bio-Refinery</b></p> <div style="display: flex; justify-content: space-between;"> <div style="text-align: left;"> <p><b>Fuels</b></p> <p>Aviation Fuel, Kerosene Gasoline, Fuel Oil</p> </div> <div style="text-align: left;"> <p><b>Petrochemicals</b></p> <p>Olefins and Aromatics for polymers, resins, adhesives, detergents, fibers, lubricants</p> </div> <div style="text-align: left;"> <p><b>Bio-fuels</b></p> <p>Ethanol, Butanol branched chain alcohols</p> </div> <div style="text-align: left;"> <p><b>Value added Bio-chemicals</b></p> </div> </div> </div> </div>	<p>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.</p>
<p style="text-align: center;"><b>Final Thoughts</b></p> <hr style="border: 1px solid purple;"/> <ul style="list-style-type: none"> <li>• Synthetic Biology is a diverse field ...</li> <li>• ... involving diverse individuals ...</li> <li>• ... working on diverse problems ...</li> <li>• ... and taking a diversity of approaches towards solving them ...</li> <li>• ... while hopefully making Biology easier to Engineer.</li> </ul>	<p>[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.</p>



## 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.

## Answers:

1. b
2. e
3. c
4. c
5. d

**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?

Answers:

1. What are some key differences between science and engineering? How does synthetic biology combine these fields?

*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. What principles of engineering are used in synthetic biology?

*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	Testing the object's ability to fulfill its function and observing any unpredicted issues.	Learning from the design → modeling → characterization process. (eg: Does this new plastic

	in the design. (eg: Does the phone slide into the case properly? Is it possible to manufacture?)	(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?)	have material properties that could be useful for something else? Does a solid vs. grid-like plastic configuration maintain protection while decreasing cost?)
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b. How are these principles applied in other areas of biology research?

*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. What are some real-world applications of synthetic biology, particularly using hosts other than yeast and bacteria?

*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. What are additional challenges to implementing synthetic biology outside of the lab?

*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?*

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## Questions on the 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?

Answers:

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.

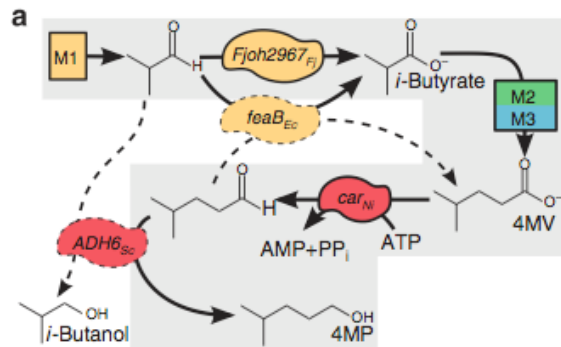
a. Substrate specificity (Fig. 2,3)

*The carboxylic acid reductase Car<sub>Ni</sub> 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<sub>EC</sub> 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.*

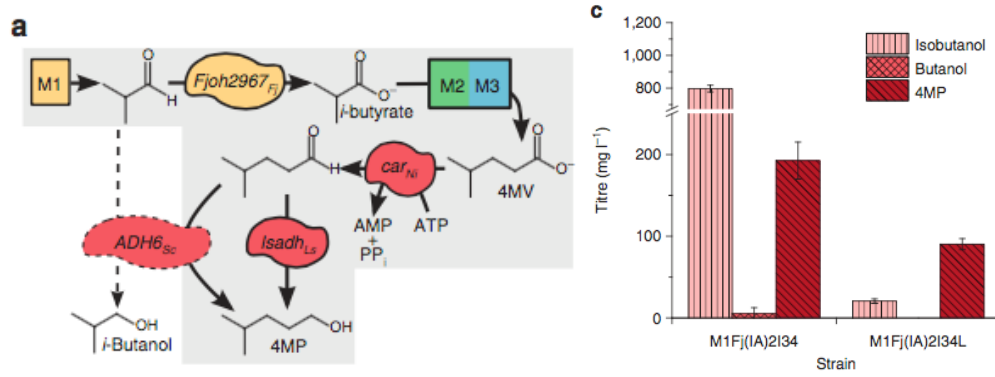
b. Avoidance of futile cycles (Fig 3)

*Since 4MV (an intermediate product) was present even when the full process was assembled, the researchers hypothesized that FeaB<sub>EC</sub> was oxidizing 4-methyl-valeraldehyde to 4MV. FeaB<sub>EC</sub> was replaced with Fjoh2967<sub>Fj</sub>, which eliminated the futile cycle and improved efficiency.*



c. Efficiency (Fig. 4)

*In module 4, ADH6<sub>sc</sub> produced large amount of the byproduct isobutanol. Replacing ADH6<sub>sc</sub> with Lsadh<sub>LS</sub> 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.