# Synthetic Biology to Create Cyanobacterial Biocatalysts for Green Chemistry

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**Synthetic biology.** Synthetic biology is a broad term with at least two fundamentally different meanings or connotations (Stephanopoulos, 2012). One is metabolic engineering on steroids, where multiple genes are deleted, introduced, or modified in an existing organism, usually with the goal of having the organism produce desired compounds or carry out desired functions. The other is –ideally- a synthetic cell or *de novo* design of an organism to carry out novel functions. Whereas there are many examples of the former type of synthetic biology, a *de novo* design of an organism has not yet been accomplished and most likely is well into the future. The reason for difficulties in *de novo* design is not that it is too challenging to chemically synthesize and assemble a large genome of 1 Mbp (1 million nucleotide pairs) or more; indeed, this has been proven to be feasible (Gibson et al., 2010). Instead, making a "new" organism *de novo* has not yet been feasible because we do not understand the intricate regulation of the cell that allows proteins to be expressed in the right amount and at the right time. Thus far, organisms with synthesized genomes have been built solely on the blueprint of existing organisms, with rather minor modifications (Gibson et al., 2010; Annaluru et al., 2014). As de novo design of viable organisms with desired functions is still outside the realm of present reality, societal discussions on this may be deferred until there is more clarity on what new life forms may be possible. Therefore, I will focus here on synthetic biology as "metabolic engineering on steroids".

**Metabolic engineering.** Molecular biology advances in the past decades have enabled the development of organisms with new functions, often to produce desired compounds. This has led to the facilitation of "green chemistry", where compounds that are typically made from petroleum can now be made biologically, thus providing a sustainable way to produce compounds that currently originate from non-renewable resources such as oil. Therefore, metabolic engineering has significant potential in making our lifestyle more sustainable.

Metabolic engineering often makes use of yeast or *Escherichia coli*, microbes that have been genetically engineered to be able to produce the appropriate products and that are grown to high cell density in large fermenters. However, in typical applications biological production with these organisms requires that one feeds the metabolically engineered organism with a fixed-carbon source such as sugar, which is converted to a desired compound, such as a building block for polymers. These sugars, directly or indirectly, come from plants that made them through photosynthesis. At large scale this is one more way that the human population uses plant material, providing competition with crops grown for food, feed or biofuel. As agricultural production will need to grow significantly in the coming decades just to keep up with increased demand for food, feed and fuel, alternate ways for sustainable production of petroleum substitutes are desired. A more direct way to provide fixed carbon for production of desired products is to have the organism perform photosynthesis itself, so that it can make the fixed carbon that it can convert into desired products (reviewed in Oliver and Atsumi, 2014). This "one-stop-shop" approach has many advantages, including that it does not divert crop production towards yet one more important use, but requires that the genetically modified organisms used for production of desired chemicals are grown outside, exposed to sunlight.

**Phototrophic production of desired compounds.** The phototrophic organisms that are used for production of "green chemicals" typically are cyanobacteria, which are photosynthetic bacteria that perform the same type of photosynthesis as plants. In fact, they are the evolutionary progenitor to chloroplasts, the photosynthetic organelles in plants. Some strains of cyanobacteria are readily transformed, and a number of strains have been developed that form desired products (Oliver and Atsumi, 2014). However, large-scale application of these organisms requires these organisms to be grown in extensive photobioreactor systems. These systems may cover large areas, and leakage etc. cannot be totally excluded. Therefore, any organisms grown there must be safe, must not persist in the environment, and must be approved for potential release. Fortunately, organisms that produce compounds for human use typically do not grow as quickly as the wild-type strain as they need to invest energy and resources into producing compounds for us. Therefore, strains that have been genetically modified in this way typically do not have the potential to become invasive species. Nonetheless, it is important to perform thorough studies regarding the safety and ecological effects to avoid potential issues.

The issue of release of genetically modified photosynthetic microbes is not fundamentally different than that of release of GMO plants. The majority of corn, soybean and cotton grown in the US is genetically modified, and there is no indication of inherent negative effects of the GMO plants during the past dozen or so years that they have been routinely planted. Any genetically modified photosynthetic microbes that are considered for large-scale production outdoors should have been demonstrated to be harmless and not particularly invasive, and any genes that have been added should not have the potential for toxicity. Genetically modified photosynthetic microbes are going through testing for EPA review of outdoor cultivation (http://www.epa.gov/biotech_rule/pubs/submiss.htm) and several strains can now be grown outdoors. The biggest differences in terms of the risks of the plant and microbial systems are (1) microbes are more prone to horizontal gene transfer, leading to potentially increased rates of gene flow from one organism to the other in microbes vs. plants, and (2) in aqueous media there is increased potential for movement and thus genetic exchange vs. in terrestrial plants, although in plants pollen has great potential for spreading as well.

Overall, society will need to weigh the advantages of metabolically engineered photosynthetic microbes in their contribution to a more sustainable society, vs. the potential risks (currently viewed to be rather minimal) that may come with inadvertent gene transfer or with issues that we currently have not considered. The potential risks related to the latter category obviously are unknown, but in view of the maturity of the field it seems unlikely that major surprises are still in store. One additional societal question to be considered in the risk/benefit analysis is what alternatives might be available and preferable to provide polymers (plastics, nylon, etc.) without the use of oil. After considering all major factors, it is my view that sustainable production of "green" chemicals in photosynthetic microbes that have been modified to produce large amounts of such compounds carries an acceptable risk and has many benefits, including reducing our oil dependence.

**References:**

Annaluru, N., et al. (2014) Total synthesis of a functional designer eukaryotic chromosome. Science 344, 55-58.

Gibson, D.G., et al. (2010) Creation of a bacterial cell controlled by a chemically synthesized genome. Science 329, 52-56.

Oliver, J.W.K., and Atsumi, S. (2014) Metabolic design for cyanobacterial chemical synthesis. Photosynth Res 120, 249-261.

Stephanopoulos, G. (2012) Synthetic biology and metabolic engineering. ACS Synth Biol 1, 514-525.