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Synthetic Biologists Spring into Action at the 245th American Chemical Society National Meeting

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ABSTRACT: As the field of synthetic biology continues to define itself, it has merged concepts from many related areas of research: molecular biology, genetics, bioengineering, and chemistry. At the 2013 Spring American Chemical Society National Meeting in New Orleans, LA, this mixture was manifested in a wealth of sessions emphasizing the use of modern synthetic biological approaches to solve many of today's biggest chemical problems. As a result of the field's diverse yet pervasive nature, synthetic biology concepts were present in several of the conferences many divisions, including Biological Chemistry, Biochemical Technology, Cellulose and Renewable Materials, and several others. Here we offer a snapshot of some of the exciting research discussed in the dedicated synthetic biology sessions throughout the week.

Chaired by Michelle Chang (U. California Berkeley) and hosted by the Division of Biological Chemistry, the official Synthetic Biology session kicked off the conference Sunday morning with several very exciting talks from top names in the field. Kristala Prather (MIT) spoke first, presenting her approach to improving production of *n*-pentanol, a biofuel and model industrial chemical.[1] She simplified a complex pathway by dividing it into several modules, which can then be further divided into individual enzymes. Feeding the cells the intermediates in these pathways can allow for parallel optimization of each module, facilitating the identification of bottlenecks and allowing transfer of modules between different final products that use similar intermediates.

Ryan Gill (U. Colorado) followed, discussing his broad vision and recent work on genome design. By introducing genomic mutations using TRMR[2] and MAGE,[3] his lab is examining the effects of multiple mutations on desired phenotypes, such as tolerance to common growth inhibitors. Huimin Zhao (U. Illinois, Urbana−Champaign) then described how his lab uses facile gene assembly techniques to build and characterize natural product biosynthetic pathways, find novel natural products, and quickly determine the enzymes responsible for each step.[4] Michelle Chang (U. California Berkeley) then complemented Professor Prather's divide and conquer approach to pathway engineering by presenting her work in the area of biofuel production; she emphasized the importance of attaining a complete molecular and biophysical understanding of each biosynthetic step and its relation to the overall pathway.[5,6] Finally Han Li from James Liao's group (U. California Los Angeles) showed some very exciting work in which different electron sources, carbon sources, and organisms were used for the direct production of biofuels from CO_2 and sunlight.[7] This could be accomplished using either natural photosynthesis processes or solar panels to reduce CO_2.

The Division of Biochemical Technology also featured several sessions focused on synthetic biology. These sessions, held over two days, featured 20 min presentations from a diverse mix of graduate students, postdocs, professors, and industry representatives. The majority of these talks focused on *in vivo* production of heterologous chemicals, with an emphasis on biofuels and value-added synthetic intermediates. These chemical production talks stressed the importance of developing replacements for petroleum-derived molecules while allowing for facile incorporation into markets built on the oil industry. Jay Keasling (U. California Berkeley, Joint Bioenergy Institute), in his Marvin J. Johnson Award lecture, discussed his lab's broad efforts at microbial production of advanced hydrocarbons.[8,9] He explained the importance of picking the correct pathway and the correct product with an emphasis on different classes of biofuels—gasoline, biodiesel, or jet fuel substitutes. To create relevant replacements for each of these, researchers in his lab have used different protein and metabolic engineering approaches to achieve correct carbon chain lengths along with branching and ring formation, while also working to enhance titers to move closer to industrially relevant levels. Many other speakers demonstrated a variety of approaches to production of biofuels and other chemicals throughout these sessions. Mattheos Koffas (Rensselaer Polytechnic Institute) talked about his focus on cofactor and pathway-intermediate control in the production of flavanones[10] and fatty acids.[11] Other talks demonstrated progress toward a variety of biofuel components such as fatty acids[12] (Micah Sheppard, Kristala Prather lab, MIT; Ting Wei Tee, Jacqueline Shanks lab, Iowa State U.), fatty alcohols[13] (Brian Pfleger, U. Wisconsin-Madison), and branched alcohols (Taek Soon Lee, JBEI) from sugar carbon sources.

While biofuels are clearly a priority in the synthetic biology community, many intermediates in the synthesis of useful products are also derived from petroleum. Production of many of these chemicals, while not required in the sheer volume of fuel replacements, represents challenges that can be met by using synthetic biology tools. Toward the goal of total petroleum replacement and greener industrial chemical production, there were several presentations on engineering

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cells to synthesize a variety of value-added products. Eric Shiue (Prather Lab, MIT) showed some recent progress in the production of glucaric acid,[14] while Gabriel Rodriguez (Shota Atsumi lab, U. California Davis) presented an interesting combination of metabolic and process engineering for the production of a toxic product, isobutyraldehyde, in *Escherichia coli*.[15] Javier Cardenas (Nancy Da Silva lab, U. California Irvine) used both standard metabolic engineering and computational tools to enhance production of triacetic acid lactone, and Matthew Wook Chang (Nanyang Technological University) outlined his lab's progress toward engineering cellular responses to the production of hydroxycinnamic acid-related molecules.[16] Finally, Himanshu Dhamankar (Prather lab, MIT) discussed production of 3-hydroxyalkanoic acids, chemicals useful as chiral building blocks for synthetic molecules.[17]

All of the aforementioned research was done using common lab strains of *E. coli* and *Saccharomyces cerevisae*; however, there were several talks throughout the conference that reminded the audience that there are countless other organisms that, while less characterized than model organisms, may naturally be a more suitable host for a variety of engineering applications. Cynthia Collins (Rensselaer Polytechnic Institute) showed her progress toward creating communication pathways between *E. coli* and *Bacillus megaterium* using modified quorum sensing pathways from Gram-positive and Gram-negative organisms. Despite the differences in these species' physiology, they were made to communicate with each other in culture.[18] Using such methods opens a broad space of potential coculture techniques wherein various organisms divide the labor for production of a final product. Ehab Ammar (Shang-Tian Yang lab, Ohio State U.) described the latest advances in the Yang lab's use of *Propionobacteria* species as a production host.[19] Sudipta Majumdar (Scott Banta lab, Columbia) and Xiaorui Yang (Shang-Tian Yang lab, Ohio State U.) shared the development of techniques for engineering of the genetically intractable organisms *Acidithiobacillus ferrooxidans* and *Clostridium cellulovorans*, respectively. Michelle O'Malley (U. California Santa Barbara) presented some interesting work on characterizing new cellulolytic organisms and enzymes from natural gut fungal isolates. In a departure from the chemical production field, Eddy Bautista (Ranjan Srivastava lab, U. Connecticut) offered a systems biology approach to *Bacillus anthracis* iron metabolism, in the quest for drug targets.

While much work has focused solely on inducing strains to produce a desired target, a few talks addressed how strains might be engineered to better handle the effects of high production of toxic chemicals and growth on non-ideal feedstocks. It is thought that growth inhibition and stress responses from these harsh conditions can actually lower yield of the desired product, so several laboratories are now turning toward engineered tolerance to a variety of inhibitors. James Winkler (Katy Kao lab, Texas A&M), for example, explored mechanisms of tolerance to acetate, a common byproduct of *E. coli* fermentations. Similarly Andrew Yongky (Wei-Shou Hu lab, U. Minnesota) presented a model of lactate production and consumption in mammalian cell culture, where fermentation pathways can lead to inhibited growth and production.[20] Danielle Tullman-Ercek (U. California Berkeley) presented our lab's progress toward achieving enhanced medium-chain alcohol tolerance in yeast, while Matthew Wook Chang (Nanyang Technological University) demonstrated that efflux pumps significantly increase yeast tolerance to medium-chain alkanes.[21] Hyun Gyu Lim (Gyoo Yeol Jung lab, POSTECH) made the case for the use of marine biomass as an abundant, lignin-free, microbial feedstock by engineering *E. coli* to grow on seaweed-derived sugars without catabolite repression.[22]

None of these metabolic engineering feats would be possible without a wide range of genetic manipulation tools at the researchers' disposal. Still, one of the major focuses of the synthetic biology field is the modification of DNA in an even more efficient and predictable manner. The development of methods for the precise control of gene expression, regulation at the gene and protein level, and prediction of resulting phenotypes is a necessity for this field to continue to progress, and several speakers offered solutions to some of these issues. Gyoo Yeol Jung (POSTECH) presented RNA regulation-based tools to help detect and isolate high producers of molecules of interest.[23] Expanding on the popular RBS Calculator software, Howard Salis (Pennsylvania State U.) discussed how his RBS Library Calculator enables predictable expression across a range of levels.[24,25] This allows for quick design of tractable libraries to find optimal expression levels of enzymes in a given metabolic pathway. Hal Alper (U. Texas), in his Daniel I.C. Wang Award lecture, described many of the tools his lab has developed for *Saccharomyces* promoter engineering.[26,27] Cameron Cotton (Jennifer Reed lab, U. Wisconsin-Madison) presented on CosMos (Continuous Modifications for Strain Design),[28] a new development on top of the Reed lab's previous software. This new software works to find the optimal genes to upregulate, downregulate, or knock out to balance production of a given chemical and biomass accumulation. Cong Trinh (U. Tennessee) discussed techniques for coupling cell growth to chemical production to optimize the production of fuels. Finally, Tirzah Glebes (Ryan Gill lab, U. Colorado) demonstrated the utility of the aforementioned TRMR genetic modification technique to develop *E. coli* strains that tolerate high levels of cellulosic hydrolysate.[29]

The broad reach and lofty goals of synthetic biology were summarized in the session's keynote lecture by William Bentley (U. Maryland). The Bentley lab's diverse research, from engineering proteins for advanced materials[30,31] to programming bacterial responses,[32] demonstrates the application of currently available genetic tools and begs the question of what future synthetic biologists will be able to accomplish. The ACS Spring Meeting, with hundreds of sessions, offered an interesting backdrop for this research and allowed scientists from many fields to share knowledge. In the future, this diversity can enhance synthetic biology to include additional basic science, biomedical, and environmental applications and powerfully demonstrate what the field has to offer.

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Notes

The authors declare no competing financial interest.

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