ACS SyntheticBiology

Viewpoint

pubs.acs.org/synthbio

4th International Conference on Biomolecular Engineering Tackles New Challenges with Synthetic Biology

Kevin V. Solomon*

Department of Chemical Engineering, University of California Santa Barbara, Santa Barbara, California 93106, United States

Engineers, biologists, and chemists from around the globe gathered for the fourth International Conference on Biomolecular Engineering (ICBE2013) in Fort Lauderdale, FL, January 13−16, 2013. Hosted by the Society of Biological Engineers, the biennial meeting serves as a platform to "cultivate knowledge and catalyze innovation" in the biological sciences. With a theme of "Molecular approaches to global challenges", ICBE2013 was a fast-paced Gordon-style conference organized by conference chairs Kristala Jones Prather (MIT) and Ali Khademhosseini (Harvard). Each day was punctuated by a keynote presentation from a pioneering visionary: George Church (Harvard), Nicholas Peppas (UT Austin), Virginia Cornish (Columbia), and James Collins (BU), who described the strides their groups have made in defining the field of biological engineering and offered their perspectives on its ability to solve problems in energy and human health. Building on these exceptional talks were invited presentations from more established researchers and reports from young investigators. Junior scientists, including graduate students and postdocs, were also offered a unique opportunity to speak to all attendees in a rapid fire session that preceded each poster session. In all, the 4 day program consisted of more than 30 research presentations across 7 sessions and roughly 100 posters covering diverse areas of research to solve key global challenges.

SCIENTIFIC MEETING OVERVIEW

Reprogramming Cells. Opening the meeting was George Church's keynote outlining the development of tools to both sequence and engineer whole genomes. Biological engineering has been fundamentally constrained by the number of biological sequences available to it. Whole genome sequencing, however, offers unparalleled opportunities in the discovery of new biologically interesting sequences for engineering applications. Advances in the past 6 years have made this capability accessible to most laboratories with a cost on the order of a few thousand dollars.[1] However, Church believes that advances in nanopore sequencing technology[2] will allow whole genome sequencing to become even more inexpensive and ubiquitous in the form of a small hand-held device. Complementing these efforts is the development of high throughput multiplexed techniques to rewrite whole genomes. With Multiplex Automated Genome Engineering (MAGE),[3] engineers can efficiently generate 4 billion microbial genomes per tube per day making "selection the new bottleneck in strain engineering". Such high throughput techniques have applications beyond traditional pathway optimization efforts in metabolic engineering, including codon reassignment in industrial organisms to implement phage resistance, genomic isolation from wildtype strains, and more diverse catalysis with orthogonal amino acids such as those with azide and ketone side chains. Current work in his group now exploits TALE nucleases[4] and CRISPR[5] to develop this capability for eukaryotic systems.

Subsequent talks by fellow synthetic biologists described efforts to engineer DNA and RNA sequences for specific applications. Chris Voigt (MIT) discussed successes in his lab replicating large Boolean complete functions within biological cells.[6] However, despite the ability to identify and tune novel circuit components, integrating components together with a predictable output still remains a challenge. To that end, in collaboration with Doug Densmore (BU), he has created a robust CAD program that will design variant circuits given only an output specification and automate their assembly, allowing the user to screen for the desired output. Similar concerns regarding the predictability of system output were voiced by Stephanie Culler (Genomatica). Leveraging -omics data, they are able to predict the strain mutations and enzyme fluxes need to optimize pathway production.[7] However, their design efforts are hampered by the variable output of characterized "parts" to tune enzyme expression. Other talks within the session were directed to the development of tools to control gene expression. Julius Lucks (Cornell) outlined the development of orthogonal antisense RNA transcriptional regulators and SHAPE-seq to probe their structure,[8,9] while Timothy Lu (MIT) described the use of Zn fingers transcription factors to engineer circuits in eukaryotic systems.[10] Charles Gersbach (Duke) similarly discussed the development of synthetic DNA-binding proteins, including Zn finger proteins and TAL effectors, which when fused to an effector such as a transcription factor, nuclease, or histone deacetylase can be used to engineer mammalian gene circuits and edit genes for disease treatment.

Engineering New Strains. Virginia Cornish (Columbia) described in her keynote a future where biological knowledge is the bottleneck to strain development and engineering. As a solution, however, she proposed high throughput *in vivo* methods of directed evolution. Addressing the problem of *in vivo* selection, she discussed the success of the yeast 3-hybrid (Y3H) system developed in her lab.[11] Using traditional synthesis techniques, Cornish's lab has developed expertise in the creation of enzyme substrates that can couple transcription factor domains with DNA binding domains. Successful cleavage (or ligation) of the substrate by the mutated enzyme leads to transcription (or silencing) of the reporter gene, directly impacting cell viability in a quantitative manner.[12,13] To introduce diversity, Cornish proposed the use of homologous

Received: January 26, 2013
Published: February 15, 2013

   dx.doi.org/10.1021/sb400007z | ACS Synth. Biol. 2013, 2, 68−71

recombination and yeast mating. Library diversity can be directly introduced by transformation of linear DNA or through the use of heritable plasmids. When coupled with mating, extraordinary diversity ($\sim 10^{13}$) can be quickly generated in a continuous manner from small libraries of $\sim 10^4$ mutants.[14] This pool size can then be rapidly screened by Y3H, alleviating the selection bottleneck.

Others discussed leveraging the power of systems biology to tackle the challenge of strain engineering. Jens Nielsen (Chalmers) described the use of genome scale metabolic models to rationally select gene targets for manipulation.[15] He, however, also agreed with Cornish that our knowledge is incomplete and argued that purely rational mutations may lead to pleiotropic effects. To fill this knowledge gap, he advocated the study of strains adaptively evolved for particular applications to better inform our systems level understanding of these pathways and improve the successes of inverse metabolic engineering.[16,17] Brian Pfleger (UW Madison) described similar systems level analyses to identify and overcome bottlenecks in saturated fatty acid production in bacteria.[18] Charlie Boon (Toronto) spoke about techniques to better understand the interconnected and pleiotropic nature of gene networks. Utilizing the concept of synthetic lethality,[19] his group has generated quantitative genetic interaction maps for 75% of all yeast genes revealing functional cross-connections across genes.[20]

Finally, a number of scientists spoke about the development of new tools for pathway and biological engineering. Cynthia Collins (RPI) discussed the development of microbial consortia to engineer more efficient pathways by minimizing the metabolic load on a given cell. Novel routes of carbon fixation (Matt Mattozzi, Harvard Wyss Institute) and enzymes for lignocellulose degradation (Michelle O'Malley, UCSB) were also described. Hal Alper (UT Austin) discussed the engineering of transporters to uptake pentoses in yeast, while Matt DeLisa (Cornell) summarized their efforts to engineer glycosylation pathways in the glycosylation-deficient *E. coli*. Similarly, novel protein tools such as allosteric switches (Marc Ostermeier, Johns Hopkins) and redox-controlled self-splicing inteins as biosensors, gene regulators, and antimicrobials (Marlene Belfort, SUNY Albany) were presented.

Molecular Approaches to Human Health. Nicholas Peppas (UT Austin) in his keynote outlined key challenges facing the delivery of protein therapeutics. His talk focused on transmucosal and oral delivery routes of protein drugs such as interferon-β for multiple sclerosis treatment through the use of nanoscale hydrogels.[21] Ravi Radhakrishnan (UPenn) described models for the design of nanocarrier surfaces for passive tissue targeting, while Yi Tang (UCLA) discussed active localized therapeutic delivery. Laura Segatori (Rice) presented on the use of nanoparticles themselves as a vector to induce autophagy in diseased cells. Finally, James Swartz (Stanford) described the use of nanoparticles as a novel and potent vaccine. Due to their cell free nature and click chemistry, virus-like particles decorated with antibodies and other antigens/proteins may be rapidly prototyped and manufactured as vaccines to a wide array of illnesses.[22] Such systems are safer and significantly reduce the production time needed to generate pandemic-level vaccine stockpiles from 200 to 14 days.

Other health-related talks centered on increased understanding of natural systems. Celeste Nelson (Princeton) described the role of mechanical gradients in the formation of branching structures such as those in the alveoli of lungs, while improved methods to empower such research were discussed by Alex Dunn (Stanford).[23] Margaret Ackerman (Dartmouth) and Peter Tessier (RPI) spoke about their efforts to better understand and tune the responses of antibodies to treat chronic conditions such as HIV infection and Alzheimer's disease. Kurt Deshayes (Genentech) described their studies to better understand the apoptotic network. While this network cannot be modulated universally across all cell lines, they have had some success developing a potent peptide ligand to induce apoptosis in some cancer lines and potentiate the efficacy of existing anticancer therapeutics.

Synthetic Biology for Regenerative Medicine and Beyond. Closing the meeting was James Collins' (BU) keynote, which summarized the evolution of synthetic biology as viewed through the prism of his lab. Growing from its infancy in transcriptional control circuits[24] to RNA regulatory devices[8,25,26] and multiplexed genetic switchboards and circuits,[6,27] Collins believes synthetic biology is poised to solve diverse nontraditional problems. Among these he cited the proliferation of engineered organisms, which has implications not only in biosecurity, but more intriguingly in that of industrial espionage. However, he believes circuits that count[28] will enable stolen or accidentally released strains to self-destruct after a preset interval unless reset. Another interesting class of problems lies in antimicrobials with applications in therapeutic biology and traditional process engineering. Collins envisions engineered bacteriophage that can potentiate existing antibiotics and resensitize resistant strains by targeting microbial DNA repair mechanisms.[29] Such phage could also express modified surface proteins, allowing for rapid diagnostics[30] or degradation of biofilms in industrial pipelines.[31] Moving forward, he believes that the tools of synthetic biology will allow for even the reengineering of humans. As examples, he discussed synthetic mRNA transfection for regenerative medicine[32] and engineered probiotics to detect and counter infections.

IN CONCLUSION

As encapsulated in the unofficial theme of the meeting, "it is no longer a question of what can be done with biology, but how quickly we can select for it". Living biological systems may be engineered to actively synthesize drugs, materials, and fuels, while biomolecules can be repurposed as biosensors and novel therapeutics and used to tune the operation of our own bodies. More importantly, our ability to engineer these systems is no longer limited by our creativity or ability to synthesize them, but rather our ability to analyze and isolate them. This rise in the recognition of the power of biology is affirmed by the growth of ICBE from its initial focus on Systems Biology, Protein Engineering, and Molecular Interactions to encompass advances in Synthetic Biology, Metabolic Engineering, Bionanotechnology, Cell & Tissue Engineering, Biophysics, Protein Engineering, and Computational Tools for Biology. With the new tools and vision presented at ICBE2013, ICBE2015 promises to be even larger, with potential solutions to an even more diverse set of global challenges.

AUTHOR INFORMATION

Corresponding Author

*E-mail: ksolomon@engineering.ucsb.edu.

Notes

The authors declare no competing financial interest.

REFERENCES

(1) Drmanac, R., Sparks, A. B., Callow, M. J., Halpern, A. L., Burns, N. L., Kermani, B. G., Carnevali, P., Nazarenko, I., Nilsen, G. B., Yeung, G., Dahl, F., Fernandez, A., Staker, B., Pant, K. P., Baccash, J., Borcherding, A. P., Brownley, A., Cedeno, R., Chen, L., Chernikoff, D., Cheung, A., Chirita, R., Curson, B., Ebert, J. C., Hacker, C. R., Hartlage, R., Hauser, B., Huang, S., Jiang, Y., Karpinchyk, V., Koenig, M., Kong, C., Landers, T., Le, C., Liu, J., McBride, C. E., Morenzoni, M., Morey, R. E., Mutch, K., Perazich, H., Perry, K., Peters, B. A., Peterson, J., Pethiyagoda, C. L., Pothuraju, K., Richter, C., Rosenbaum, A. M., Roy, S., Shafto, J., Sharanhovich, U., Shannon, K. W., Sheppy, C. G., Sun, M., Thakuria, J. V., Tran, A., Vu, D., Zaranek, A. W., Wu, X., Drmanac, S., Oliphant, A. R., Banyai, W. C., Martin, B., Ballinger, D. G., Church, G. M., and Reid, C. A. (2010) Human genome sequencing using unchained base reads on self-assembling DNA nanoarrays. *Science 327*, 78−81.

(2) Kumar, S., Tao, C., Chien, M., Hellner, B., Balijepalli, A., Robertson, J. W. F., Li, Z., Russo, J. J., Reiner, J. E., Kasianowicz, J. J., and Ju, J. (2012) PEG-labeled nucleotides and nanopore detection for single molecule DNA sequencing by synthesis. *Sci. Rep. 2*, 684.

(3) Wang, H. H., Isaacs, F. J., Carr, P. A., Sun, Z. Z., Xu, G., Forest, C. R., and Church, G. M. (2009) Programming cells by multiplex genome engineering and accelerated evolution. *Nature 460*, 894−898.

(4) Briggs, A. W., Rios, X., Chari, R., Yang, L., Zhang, F., Mali, P., and Church, G. M. (2012) Iterative capped assembly: Rapid and scalable synthesis of repeat-module DNA such as TAL effectors from individual monomers. *Nucleic Acids Res. 40*, e117−e117.

(5) Mali, P., Yang, L., Esvelt, K. M., Aach, J., Guell, M., DiCarlo, J. E., Norville, J. E., and Church, G. M. (2013) RNA-guided human genome engineering via Cas9. *Science*, DOI: DOI:10.1126/science.1232033.

(6) Moon, T. S., Lou, C., Tamsir, A., Stanton, B. C., and Voigt, C. A. (2012) Genetic programs constructed from layered logic gates in single cells. *Nature 491*, 249−253.

(7) Yim, H., Haselbeck, R., Niu, W., Pujol-Baxley, C., Burgard, A., Boldt, J., Khandurina, J., Trawick, J. D., Osterhout, R. E., Stephen, R., Estadilla, J., Teisan, S., Schreyer, H. B., Andrae, S., Yang, T. H., Lee, S. Y., Burk, M. J., and Dien, S. V. (2011) Metabolic engineering of Escherichia coli for direct production of 1,4-butanediol. *Nat. Chem. Biol. 7*, 445−452.

(8) Lucks, J. B., Qi, L., Mutalik, V. K., Wang, D., and Arkin, A. P. (2011) Versatile RNA-sensing transcriptional regulators for engineering genetic networks. *Proc. Natl. Acad. Sci. U.S.A. 108*, 8617−8622.

(9) Lucks, J. B., Mortimer, S. A., Trapnell, C., Luo, S., Aviran, S., Schroth, G. P., Pachter, L., Doudna, J. A., and Arkin, A. P. (2011) Multiplexed RNA structure characterization with selective 2′-hydroxyl acylation analyzed by primer extension sequencing (SHAPE-Seq). *Proc. Natl. Acad. Sci. U.S.A. 108*, 11063−11068.

(10) Khalil, A. S., Lu, T. K., Bashor, C. J., Ramirez, C. L., Pyenson, N. C., Joung, J. K., and Collins, J. J. (2012) A synthetic biology framework for programming eukaryotic transcription functions. *Cell 150*, 647−658.

(11) Baker, K., Bleczinski, C., Lin, H., Salazar-Jimenez, G., Sengupta, D., Krane, S., and Cornish, V. W. (2002) Chemical complementation: A reaction-independent genetic assay for enzyme catalysis. *Proc. Natl. Acad. Sci. U.S.A. 99*, 16537−16542.

(12) Lin, H., Tao, H., and Cornish, V. W. (2004) Directed evolution of a glycosynthase via chemical complementation. *J. Am. Chem. Soc. 126*, 15051−15059.

(13) Peralta-Yahya, P., Carter, B. T., Lin, H., Tao, H., and Cornish, V. W. (2008) High-throughput selection for cellulase catalysts using chemical complementation. *J. Am. Chem. Soc. 130*, 17446−17452.

(14) Romanini, D. W., Peralta-Yahya, P., Mondol, V., and Cornish, V. W. (2012) A heritable recombination system for synthetic Darwinian evolution in yeast. *ACS Synth. Biol 1*, 602−609.

(15) Liu, L., Agren, R., Bordel, S., and Nielsen, J. (2010) Use of genome-scale metabolic models for understanding microbial physiology. *FEBS Lett. 584*, 2556−2564.

(16) Hong, K.-K., Vongsangnak, W., Vemuri, G. N., and Nielsen, J. (2011) Unravelling evolutionary strategies of yeast for improving galactose utilization through integrated systems level analysis. *Proc. Natl. Acad. Sci. U.S.A. 108*, 12179−12184.

(17) Hong, K.-K., and Nielsen, J. (2012) Recovery of phenotypes obtained by adaptive evolution through inverse metabolic engineering. *Appl. Environ. Microbiol. 78*, 7579−7586.

(18) Lennen, R. M., Kruziki, M. A., Kumar, K., Zinkel, R. A., Burnum, K. E., Lipton, M. S., Hoover, S. W., Ranatunga, D. R., Wittkopp, T. M., Marner, W. D., and Pfleger, B. F. (2011) Membrane stresses induced by overproduction of free fatty acids in Escherichia coli. *Appl. Environ. Microbiol. 77*, 8114−8128.

(19) Tucker, C. L., and Fields, S. (2003) Lethal combinations. *Nat. Genet. 35*, 204−205.

(20) Costanzo, M., Baryshnikova, A., Bellay, J., Kim, Y., Spear, E. D., Sevier, C. S., Ding, H., Koh, J. L. Y., Toufighi, K., Mostafavi, S., Prinz, J., Onge, R. P. S., VanderSluis, B., Makhnevych, T., Vizeacoumar, F. J., Alizadeh, S., Bahr, S., Brost, R. L., Chen, Y., Cokol, M., Deshpande, R., Li, Z., Lin, Z.-Y., Liang, W., Marback, M., Paw, J., Luis, B.-J. S., Shuteriqi, E., Tong, A. H. Y., Dyk, N., van, Wallace, I. M., Whitney, J. A., Weirauch, M. T., Zhong, G., Zhu, H., Houry, W. A., Brudno, M., Ragibizadeh, S., Papp, B., Pál, C., Roth, F. P., Giaever, G., Nislow, C., Troyanskaya, O. G., Bussey, H., Bader, G. D., Gingras, A.-C., Morris, Q. D., Kim, P. M., Kaiser, C. A., Myers, C. L., Andrews, B. J., and Boone, C. (2010) The genetic landscape of a cell. *Science 327*, 425−431.

(21) Kamei, N., Morishita, M., Chiba, H., Kavimandan, N. J., Peppas, N. A., and Takayama, K. (2009) Complexation hydrogels for intestinal delivery of interferon β and calcitonin. *J. Controlled Release 134*, 98−102.

(22) Ng, P. P., Jia, M., Patel, K. G., Brody, J. D., Swartz, J. R., Levy, S., and Levy, R. (2012) A vaccine directed to B cells and produced by cell-free protein synthesis generates potent antilymphoma immunity. *Proc. Natl. Acad. Sci. U.S.A. 109*, 14526−14531.

(23) Borghi, N., Sorokina, M., Shcherbakova, O. G., Weis, W. I., Pruitt, B. L., Nelson, W. J., and Dunn, A. R. (2012) E-cadherin is under constitutive actomyosin-generated tension that is increased at cell−cell contacts upon externally applied stretch. *Proc. Natl. Acad. Sci. U.S.A. 109*, 12568−12573.

(24) Gardner, T. S., Cantor, C. R., and Collins, J. J. (2000) Construction of a genetic toggle switch in Escherichia coli. *Nature 403*, 339−342.

(25) Isaacs, F. J., Dwyer, D. J., Ding, C., Pervouchine, D. D., Cantor, C. R., and Collins, J. J. (2004) Engineered riboregulators enable post-transcriptional control of gene expression. *Nat. Biotechnol. 22*, 841−847.

(26) Bayer, T. S., and Smolke, C. D. (2005) Programmable ligand-controlled riboregulators of eukaryotic gene expression. *Nat. Biotechnol. 23*, 337−343.

(27) Callura, J. M., Cantor, C. R., and Collins, J. J. (2012) Genetic switchboard for synthetic biology applications. *Proc. Natl. Acad. Sci. U.S.A. 109*, 5850−5855.

(28) Bonnet, J., Subsoontorn, P., and Endy, D. (2012) Rewritable digital data storage in live cells via engineered control of recombination directionality. *Proc. Natl. Acad. Sci. U.S.A. 109*, 8884−8889.

(29) Lu, T. K., and Collins, J. J. (2009) Engineered bacteriophage targeting gene networks as adjuvants for antibiotic therapy. *Proc. Natl. Acad. Sci. U.S.A. 106*, 4629−4634.

(30) Willford, J. D., Bisha, B., Bolenbaugh, K. E., and Goodridge, L. D. (2011) Luminescence based enzyme-labeled phage (Phazyme) assays for rapid detection of Shiga toxin producing Escherichia coli serogroups. *Bacteriophage 1*, 101−110.

(31) Lu, T. K., and Collins, J. J. (2007) Dispersing biofilms with engineered enzymatic bacteriophage. *Proc. Natl. Acad. Sci. U.S.A. 104*, 11197−11202.

(32) Warren, L., Manos, P. D., Ahfeldt, T., Loh, Y.-H., Li, H., Lau, F., Ebina, W., Mandal, P. K., Smith, Z. D., Meissner, A., Daley, G. Q., Brack, A. S., Collins, J. J., Cowan, C., Schlaeger, T. M., and Rossi, D. J. (2010) Highly efficient reprogramming to pluripotency and directed

differentiation of human cells with synthetic modified mRNA. *Cell Stem Cell 7*, 618−630.

dx.doi.org/10.1021/sb400007z | *ACS Synth. Biol.* 2013, 2, 68−71