SYMPOSIUM GI03

Synthetic Biology—An Accelerator of Materials Research and Development
November 26 - November 27, 2018

Symposium Organizers
Wendy Crookes-Goodson, Air Force Research Laboratory
Michael Jewett, Northwestern University
Petra Oyston, Porton Down
Melissa Rhoads, Lockheed Martin

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* Invited Paper

SESSION GI03.01: Synthetic Biology—High-Throughput Capabilities and 3D Printing
Session Chairs: Sarah Glaven and Melissa Rhoads
Monday Morning, November 26, 2018
Hynes, Level 1, Room 109

8:30 AM WELCOME AND INTRODUCTION BY MELLISA RHOADS, LOCKHEED MARTIN SPACE SYSTEMS

9:00 AM *GI03.01.02
A Sequence-to-Molecules and Materials Pipeline—Enabling Everything from 1-hexadecanol to Vincristine Ishtiaq Saaem[1, 2, 3]; [1]The Foundry, Cambridge, Massachusetts, United States; [2]Broad Institute of MIT and Harvard, Cambridge, Massachusetts, United States; [3]Synthetic Biology Center, Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts, United States.

Centralized facilities for genetic engineering, or "biofoundries", offer the potential to design organisms to address emerging needs in medicine, agriculture, industry, and defense. To date, by applying a diversity of new approaches, we have produced the desired molecule or material that has been asked by clients from the pharmaceutical and biotech industries as well as from the DOD. Specifically, we increased the titers of 1-hexadecanol, pyrrolnitrin, pacidamycin D, and clavulanic acid, found novel routes to the enediyne warhead underlying powerful antimicrobials, established a cell-free system for monoterpene production, and produced an intermediate toward vincristine biosynthesis. Furthermore, we have produced small-molecules linalool and phloroglucinol that, respectively, serve as precursors to JP-10 that fuels missiles and TATB that detonates the warheads. For materials production, we have encoded 7802 individually retrievable pathways to 540 bisindoles in a DNA pool, and created 61 biosynthetic capsaicin variants that could be central to high-performance polymers. In sum, we constructed several megabases of DNA, built hundreds of strains spanning six species (Saccharomyces cerevisiae, Escherichia coli, Streptomyces albidoflavus, Streptomyces coelicolor, Streptomyces clavuligerus and Streptomyces albovinaceus), established two cell-free systems, and performed nearly a thousand assays developed in-house for the molecules.

9:30 AM GI03.01.03
Synthetic Biology for Protein-Based Materials Fuzhong Zhang; Energy, Environmental & Chemical Engineering, Washington University, Saint Louis, Missouri, United States.

Advances in synthetic biology have enabled the quantitative engineering of dynamic biological processes for the precise control of cellular reaction networks, allowing engineered cells to perform complex tasks and to produce various small molecules, which can be used as biofuel replacements, polymer precursors, nutraceuticals, and pharmaceuticals. Compared to small molecules, the production of macromolecular biomaterials by synthetic biology approaches is strongly lagging. Nature has evolved various types of protein materials with remarkable mechanical properties and functions. To facilitate the practical use of these materials, we aim to engineer bacterial cell factories to overproduce high-performance protein-based materials, such as spider silks. We have developed synthetic biology strategies that allow us to synthesize ultra-high molecular weight spidroins (556 kDa) at gram per liter scales. Fibers spun from our microbially produced spidroin fully replicate the mechanical performance of natural spider silk by all common metrics, i.e. tensile strength (1.03 ± 0.11 GPa), modulus (13.7 ± 3.0 GPa), extensibility ($18 \pm 6\%$), and toughness (114 ± 51 MJ/m^3). The developed strategy reveals a path to more dependable production of high-performance silks for mechanically-demanding applications while also providing a platform to facilitate production of other high-performance natural materials.

9:45 AM GI03.01.04
Lipid Droplet Microarrays for High Throughput Screening in Synthetic Biology Aubrey Kusi-Appiah and Steven Lenhert; Biological Science, Florida State University, Tallahassee, Florida, United States.

A current obstacle in the use of synthetic biology for materials synthesis and discovery is the high throughput characterization of novel materials produced by biological organisms. Furthermore, identifying the environmental conditions that allow biological cells to produce the desired materials is challenging due to the large amount of possible cell culture ingredients and conditions. High throughput screening is currently carried out in the pharmaceutical industry for this purpose, but is cost prohibitive for many materials applications. Miniaturization of high throughput screening promises to lower the cost and allow portable screens suitable for academic labs. We have developed a novel screening platform compatible with standard cell culture techniques that is scalable to a screen of 50,000 different materials on the area of a single titer plate using cell-based assays.[1] The technology is based on lipid droplet microarrays on surfaces where the droplets encapsulate different compounds or nanomaterials. Development of this technology required solving several

technical challenges, including scalable fabrication,[2] quantification of the dosage delivered from the droplets,[3] and most recently chemical processing to ensure stability upon immersion into aqueous solution.[4] The technology is now ready to be applied to high throughput screening in synthetic biology.

References:
[1] A. E. Kusi-Appiah, N. Vafai, P. J. Cranfill, M. W. Davidson, S. Lenhert, Biomaterials 2012, 33, 4187.
[2] T. W. Lowry, A. Kusi-Appiah, J. J. Guan, D. H. Van Winkle, M. W. Davidson, S. Lenhert, Advanced Materials Interfaces 2014, 1, 1300127.
[3] A. E. Kusi-Appiah, T. W. Lowry, E. M. Darrow, K. A. Wilson, B. P. Chadwick, M. W. Davidson, S. Lenhert, Lab Chip 2015, 15, 3397.
[4] A. E. Kusi-Appiah, T. W. Lowry, N. Vafai, D. H. Van Winkle, S. Lenhert, 2017 Fluid Lipid Multilayer Stabilization by Tetraethyl Orthosilicate for Underwater AFM Characterization and Cell Culture Applications. *MRS Advances* 2, 3553-3558.

10:00 AM BREAK

10:30 AM *GI03.01.05
3D Printing of Engineered Bacteria for the Production of Patterned Living Materials Anne S. Meyer; University of Rochester, Rochester, New York, United States.

In order to create crisp, defined patterns of biologically-created materials, new technologies need to be developed and implemented. The Meyer lab is developing first-of-their-kind bacterial 3D printers that can deposit engineered bacteria in specific three-dimensional patterns using simple devices and chemistries. Our bacterial 3D printers have fully automated, coordinated control of the pumps and printhead, allowing for high spatial resolution (<mm-scale) printing of bacteria onto wet or dry surfaces. Our printers mix an alginate-containing bacterial culture with a calcium chloride solution upon printing, triggering cross-linking of the alginate molecules to form a stable, biocompatible scaffold to support the bacteria. After printing, the scaffold is removable by dissolving the cross-linked alginate matrix with sodium citrate. We are applying our printer to the fabrication of engineered biofilms, groups of bacteria that live within a spatially structured polymer matrix. We use our 3D printer to deposit engineered *E. coli* that are able to produce CsgA fibrils, the major protein component of biofilm polymer matrices. These engineered bacteria can stick to the printing surface even after the printing scaffold has been dissolved away, creating free-standing, patterned biofilms. These model biofilms will be crucial for future development of anti-biofilm strategies, for which no reproducible model biofilm test system is currently available, as well as for the reliable production of beneficial living materials, which could be applied for water and soil purification or mineral extraction.

11:00 AM GI30.01.06
Bacterial Foundry of the Self-Regenerating and Functional Materials Anna Duraj-Thatte, Avinash M. Basavanna, Arjirios Sourlis and Neel Joshi; Harvard University, Boston, Massachusetts, United States.

Protein-based materials have been successfully used for several applications in nanotechnology, bioengineering and medicine. Their diverse biofunctions and unique structures make them ideal building blocks to create large scale functional materials. However, there are several challenges: scalable production, isolation, expensive synthesis and processing keep recombinant protein- based materials away from other most commercially successful large-scale living materials such as cellulose or mycelium-based materials.
To tackle this problem, we developed a novel method for the rapid biosynthesis of tailored hydrogels in a single step, directly from bacterial culture, using a protocol that requires no protein purification. The hydrogel scaffold is based on the engineered extracellular matrix protein CsgA, which self-assembles into a fibrous mesh-like network that is 4-7% of hydrogel mass.
Most importantly, by genetic engineering of CsgA protein we can modulate a range of rheological properties as well as incorporate any specific functional modules for desired applications. This unique method leaded us to create a novel viscoelastic and shear-thinning 3D printing inks that are entirely biofabricated by genetically engineered bacteria. Due to biofabrication process that we developed, this hydrogel can contain living bacteria capable of producing more living ink or living bacteria can be removed during fabrication. This novel bacterial ink can be used for printing complex large scale materials with specific functions.

11:15 AM GI03.01.07
3D Printing of Bacteria to Create Living Materials Patrick A. Rühs; Department of Bioengineering, University of California, Berkeley, Berkeley, California, United States.

Despite recent advances to control the spatial composition and dynamic functionalities of bacteria embedded in materials, bacterial localization into complex 3D geometries remains a major challenge. Here we demonstrate a 3D printing approach to create bacteria-derived functional materials by combining the natural diverse metabolism of bacteria with the shape design freedom of additive manufacturing (1).
For 3D printing we use a recently developed multimaterial direct ink writing technique (2) which allows us to incorporate bacteria in biocompatible inks within the same 3D printed material. Our bioinks are designed by combining different hydrogels to form a paste-like ink, which after printing is crosslinked by low intensity UV light. To obtain accurate 3D printed structures, we determine the ideal rheological properties prior, during and after printing, demonstrating the effect of the printing steps on the bioink. With this approach we are able to obtain a hydrogel which supports bacteria growth while still maintaining a high shape fidelity in 3D printing.
We embedded bacteria in the biocompatible and functionalized 3D printing ink and printed two types of 'living materials' capable of degrading pollutants and of producing medically relevant bacterial cellulose. Furthermore, we demonstrate that bacteria proliferation is a function of viscosity and oxygen availability. By fine-tuning the single ink components, we adjust the viscosity to match the growth profile of our cells. With this printing platform, we envision the use of additive manufacturing materials combined together with cells to be used for new and biomedical applications.

(1) Schaffner, M.*, Rühs, P.A.*, Coulter, F., Kilcher, S., Studart, A.R. 3D printing of bacteria into functional complex materials, Science Advances, Vol. 3, no. 12, eaao6804 (2017)
(2) Kokkinis, D., Schaffner, M. & Studart, A. R. Multimaterial magnetically assisted 3D printing of composite materials. Nature Communications, 6, (2015).

11:30 AM *GI03.01.08
Living Conductive Materials for the Marine Environment Sarah M. Glaven[3], Lina J. Bird[1], Elizabeth J. Onderko[1], Daniel Phillips[2], Matthew Yates[3] and Christopher A. Voigt[4]; [1]National Research Council, Washington, District of Columbia, United States; [2]American Society for Engineering Education, Washington, District of Columbia, United States; [3]U.S. Naval Research Laboratory, Washington, District of Columbia, United States; [4]Massachusetts Institute of Technology, Cambridge, Massachusetts, United States.

It is well established that metal-respiring bacteria, such as *Geobacter* and *Shewanella,* perform extracellular electron transfer (EET) when grown as

biofilms on the surface of electrodes. The electron transport (ET) proteins that enable this microbial electrical wiring in *Shewanella* have been identified and successfully expressed in *E. coli*, conferring an increase in the amount of current produced over the wild type background. The ability to rationally engineer EET processes in electrochemically active biofilms could result in leap-ahead technological advancements in biomaterials applications including microbial electrosynthesis, bioremediation, and microbial bioelectronics, specifically under austere conditions, such as the ocean. However, these applications are currently limited by a lack of understanding of the physiological constraints of the host bacterium (chassis) to properly and predictably express and orient ET proteins (e.g. *c*-type cytochromes) in the cell membrane, the ability to rapidly screen a large number of constructs for different ET pathways, and a library of operationally relevant chassis strains. In this talk I will describe results demonstrating the use of a suite of highly-optimized small molecule sensors (Marionette) developed for control over *E. coli* cellular processes to control expression of the *Shewanella* MtrCAB pathway, and accessory electron carriers, in *Marinobacter atlanticus*. First, Marionette sensors were transformed into *M. atlanticus*and assessed for expression of yellow fluorescent protein (YFP) after the addition of 7 different small molecules (choline, vanillin, naringenin, DAPG, cumate, tetracycline, and IPTG) during both planktonic growth and in the biofilm state. For most sensors, a broad dynamic range was observed similar to that demonstrated with *E. coli* when fluorescence was measured during log phase growth. Increasing fluorescence was also observed over time in biofilm associated cells as long as growth medium with small molecule inducer was continuously refreshed. When YFP was replaced with ET proteins, expression of MtrCAB led to an increase in current compared to the wild type strain when induced prior to inoculation into a bioelectrochemical system (BES). However, the effect was not robust enough for biosensing. Moving the MtrCAB pathway from a plasmid construct to the chromosome enabled more control over the quantity of protein expressed, however, no improvement in current was observed. When the same construct was tested in *Shewanella oneidensis* MR1 lacking the native MtrCAB pathway, current was found to be inducible following biofilm formation. Based on these results, we conclude that although the MtrCAB pathway can be successfully expressed in *M. atlanticus*, further optimization of export of these proteins to the outer membrane and/or connection to the inner membrane electron pool may be necessary. Understanding these constraints will advance the development of engineered bio-electrochemically active biofilms for development of self-healing living materials for energy and next generation electronics for the marine environment.

SESSION GI03.02: Synthetic Biology—Aerospace Applications, Metals and Composites
Session Chairs: Anne Meyer and Petra Oyston
Monday Afternoon, November 26, 2018
Hynes, Level 1, Room 109

1:30 PM *GI03.02.01
Synthetic Biology for Aerospace Materials Maneesh K. Gupta; Materials and Manufacturing Directorate, Air Force Research Laboratory, Wright-Patterson AFB, Ohio, United States.

The DOD currently faces a broad range of materials challenges. The changing geopolitical environment has created a need for agile supply chains of critical materials and components. The push for miniaturization and light-weighting has created a need for precise and scalable assembly of materials with limited defects. Additionally, the diverse and challenging operational environments faced by the warfighter has created a need for multifunctional materials that can sense and respond to the human and the surrounding environment. Strategic utilization of synthetic biology has the potential to provide new materials capabilities that can address many of these critical challenges. This talk will highlight work being done in synthetic biology at the Air Force Research Laboratory, Materials and Manufacturing Directorate to enable new solutions to these challenges. Specifically, new strategies and pipelines for the discovery and engineering of novel enzymes and proteins that can be used in the production of critical material components will be discussed.

2:00 PM *GI03.02.02
The Biological Upcycling of Metals Louise Horsfall; University of Edinburgh, Edinburgh, United Kingdom.

Microorganisms have the potential to manufacture metallic nanoparticles, irrespective of the source of metal cations, and provide us with new particles with novel functions. To exploit this we are identifying and optimising genetic elements with an aim to increase nanoparticle production and control nanoparticle size and homogeneity; in effect standardising nanoparticle samples by using biology. Whilst developing this process we are exploring its application in the treatment of contaminated waste, water and land. For the former application we are working to remove copper from whisky distillery by-products and for the latter application we are part of a larger collaboration aiming to financially incentivise land decontamination. After using phytoremediation to hyperaccumulate metal contaminants from the soil, plants are harvested, processed and used as a source of metals for bacterial nanoparticle synthesis. Both applications illustrate how synthetic biology might contribute to moving us towards a more sustainable circular economy.

2:30 PM *GI03.02.03
Living Foundations—Using Synthetic Biology to Hierarchically Assemble Structural Materials Caroline Ajo-Franklin[1], Marimikel Charrier[1], Dong Li[1], Sneha Jani[1], Victor Mann[1], Behzad Rad[1], Bruce E. Cohen[1], Kathleen Ryan[2] and Paul Ashby[1]; [1]Lawrence Berkeley National Laboratory, Berkeley, California, United States; [2]Dept of Plant and Microbial Biology, University of California, Berkeley, Berkeley, California, United States.

Structural materials synthesized by organisms, such as bones, shells, and wood, exhibit remarkable mechanical properties due to their hierarchical assembly of hard and soft components across the nanometer to the micron scales. While engineering analogs to these materials would open new frontiers, there is currently no route to mimic the 2D hierarchical ordering of natural composites. Here we lay the foundations for bottom-up assembly of engineered living-material composites along the cell body using a synthetic biology approach and demonstrate the hierarchical assembly of these composites confers switchable mechanical properties. We engineer the surface-layer (S-layer) of *Caulobacter crescentus* to display peptides that permit covalent attachment of proteins, nanocrystals, and protein-based polymers to the extracellular surface without additional engineering. This cell surface binding is uniform, specific, and covalent, and its density can be controlled based on the location of the insertion within the S-layer. Using this platform, we construct composite materials composed of living *C. crescentus* cells crosslinked by nanocrystals. These ‘hybrid bacterial spheroids’ are 30 times more stiff than cell-nanocrystal composites lacking these crosslinks. Additionally, the stiffness of these composites can be changed dynamically by breaking the cell-nanocrystal crosslinking. Taken together, this work provides a platform for creating hierarchically-assembled living materials with switchable mechanical properties.

3:00 PM BREAK

3:30 PM PANEL DISCUSSION: AN ACCELERATOR OF MATERIALS RESEARCH AND DEVELOPMENT

SESSION GI03.04: Synthetic Biology—High Performing Proteins and Material Testing
Session Chairs: Wendy Crookes-Goodson and Maneesh Gupta
Tuesday Morning, November 27, 2018
Hynes, Level 1, Room 109

8:30 AM *GI03.04.01
High Throughput Methods for Accelerating Materials Synthesis with Synthetic Biology Milan Mrksich; Department of Biomedical Engineering, and Center for Synthetic Biology, Northwestern University, Evanston, Illinois, United States.

This talk will describe an approach for using mass spectrometry and arrays of self-assembled monolayers to perform quantitative experiments in high throughput. The arrays are prepared by immobilizing small molecules, proteins, peptides and carbohydrates to self-assembled monolayers of alkanethiolates on gold. This arrays are then treated with reactants—either chemical reagents or enzymes—and then analyzed using the SAMDI technique to identify the masses of substituted alkanethiolates in the monolayer and therefore a broad range of reactivities and post-translational modifications—including kinase, protease, methyltransferase and carbohydrate-directed modifications—and for discovering chemical reactions. This talk will describe applications to high throughput experiments, including the profiling of biosynthetic pathways, the use of carbohydrate arrays to discover novel enzymes, the preparation of peptide arrays to profile the enzyme activities in cell lysates and high-throughput screening to discover novel reactions and small molecular modulators. These examples illustrate the broad capability of the SAMDI method to accelerate the design-build-test cycle for materials.

9:00 AM *GI03.04.02
Repurposing the Translation Apparatus for Synthetic Biology and Materials Design—Engineering Nanomaterials from Marine Biopolymers Jasmine M. Hershewe[1, 2], Chelsea C. Buck[3, 4], Patrick B. Dennis[3] and Michael Jewett[1, 2]; [1]Department of Chemical and Biological Engineering, Northwestern University, Evanston, Illinois, United States; [2]Center for Synthetic Biology, Northwestern University, Evanston, Illinois, United States; [3]Air Force Research Laboratory, Dayton, Ohio, United States; [4]Department of Materials Science, University of Dayton, Dayton, Ohio, United States.

Nature has evolved exquisite systems that serve as inspiration for the design of synthetic materials with user-defined functions and properties. DNA-encoded proteins comprise many of these materials, and the molecular assembly of protein subunits into hierarchical structures is responsible for some of the most essential, sophisticated, and diverse functions in nature. With the cost of DNA synthesis quickly falling to < $0.05/base, engineering materials using synthetic biology has emerged as an interesting, scalable avenue for advancing nanobiotechnology and materials science.

Key hurdles that exist for engineering and discovering tunable protein materials are the lengthy design, build, test cycles associated with building proteins in living cells. Synthetic biology has enabled the development of several key platform technologies that could accelerate biomaterials design at the 'build' stage of the cycle. In particular, we have developed a high-yielding cell-free protein synthesis platform from *Escherichia coli* that synthesizes up to 1.8g/L of protein in batch mode in ~8 hours. With an eye toward enhancing the chemical diversity of protein biopolymers, we have recently interfaced this platform with orthogonal translation systems (OTS) to genetically encode non-canonical amino acids (ncAAs) into proteins. We have demonstrated the ability to site-specifically introduce multiple identical ncAAs into a single protein (>10) with high titers and purity.

Using this foundation, we have begun studying a promising target for engineering protein-based materials, sourced from the giant squid, called 'suckerins.'

Suckerins are a class of structural proteins that form sucker ring teeth assemblies that display robust mechanical properties and thermoplastic behavior. We synthesized and purified milligram quantities of a 23kDa suckerin isoform, 'suckerin-12,' bearing the ncAA para-L-azido phenylalanine (pAzF). We systematically investigated the ion- and pH-responsiveness of suckerin-12 and exploited the biochemistry of the molecule to assemble protein core nanoparticles with tuneable diameters between tens to hundreds of nanometers. We have begun utilizing the incorporated ncAA to functionalize suckerin-12 with hydrophilic ligands to generate synthetic, conjugate nanomaterials with altered self-assembly properties. We anticipate this work will be useful for elucidating important design parameters for fabricating protein-based nanoparticles and, more broadly, for accelerated engineering of protein nanomaterials using synthetic biology.

9:30 AM *GI03.04.03
Increasing Throughput for the Development of Protein-Based Materials Bradley Olsen; MIT, Cambridge, Massachusetts, United States.

Proteins have emerged as a very attractive chemistry for the development of new materials, ranging from elastomers to biocatalysts to photonics to biomaterials. The application of synthetic biology at the interface of polymer science enables their design and engineering into such a wide variety of structures by using genetic engineering to control primary sequence and therefore impact all other hierarchical levels of structure. However, compared with the vast size of design space for protein materials, only a small number have been extensively explored. Originally, the limitation was on the production of genes encoding for different materials, which were often difficult to synthesize due to their large sizes and highly repetitive nature. With advances in gene synthesis, the limitation has moved to the ability to effectively express, purify, and test a large number of materials.

Unlike the engineering of enzymes or molecular binders, protein materials only demonstrate useful function in a purified state, posing new challenges for increasing throughput in this area of synthetic biology. Here, we talk about several different perspectives on this challenge. First, we examine methods for the simultaneous purification of hundreds of different protein materials using elastin-like protein tag technology originally developed by Chilkoti. With careful engineering of the system, combined with key advances in ELP thermodynamics that eliminate steps that are traditionally difficult or expensive to execute in high throughput, we are able to show the purification of materials in a series of well plates and demonstrate their effective evaluation.

Second, using the Opentrons low cost, open-source automation platform, we have developed methods to automate key steps and assays in clonal design, selection, and protein evaluation. While strain engineering is often a key step in industrial protein development, this is rarely performed in academic protein material design, potentially introducing a key bottleneck in materials yield. By using different optical designs, the need for integrating a plate reader with the automated system can be eliminated for many assays, providing an extremely low-cost and widely accessible platform that can increase throughput and improve access by the community to protein materials engineering.