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Programming bacteria to grow novel materials

Monday August 15th
Lecture 4:00-5:00 p.m. | Physics/Astronomy Auditorium (PAA) A118
Reception 5:00-6:00 p.m. | Benson Hall Lobby

Bio

Dr. Sara Molinari started her studies in Italy with a Bachelor in Pharmaceutical Biotechnology at University of Perugia and a Master in Bioinformatics at University of Milano-Bicocca. She joined the System, Synthetic and Physical Biology PhD program at Rice University and graduated from Matthew Bennett's Lab. Her PhD was largely funded by the DARPA ELM program that organized pioneer research on engineered living materials. She graduated with a thesis on engineering differentiation in bacterial cells to enable the creation of multicellular systems from prokaryotic hosts. Now she is a postdoctoral researcher in Caroline Ajo-Franklin's lab, where she successfully created the first example macroscopic de novo engineered living material that grows from genetically modified bacteria.

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Abstract

A de novo matrix for macroscopic living materials from bacteria

Dr. Sara Molinari

Artificial materials have always been central to technological advancements, from steel during the industrial revolution to engineered semiconductors in the digital era. Although there exists a ceaseless demand for new materials to support human progress, pressing environmental challenges, such as rising global temperatures and dwindling natural resources, impose upon us the need for eco-friendly production practices. Biological organisms integrate performance and sustainability by creating living materials, such as skin, bone, wood or biofilms. They combine remarkable physical and biological properties with the ability to self-assemble from basic nutrients under mild conditions, self-repair in case of damage, and dynamically respond to external stimuli[1]. Engineered living materials (ELMs)[2–4] aspire to emulate the characteristics of their natural counterparts, but with the additional goal to also incorporate non-natural biological functions and tailored mechanical properties. ELMs are composites of microorganisms embedded in a biopolymer matrix, where cells define the biological activity, while the matrix controls the bulk material composition and structure[5]. For this reason, engineering macroscopic ELMs that grow from the bottom-up with a synthetic biomolecular matrix will allow control over their mechanical properties. However, we have lacked the tools and design rules to genetically encode a synthetic matrix that programs collective cell self-organization into macroscopic structures[5]. As result, most ELMs are microscopic[6–10] and must be processed into macroscopic materials. The few macroscopic ELMs have been created by genetically modifying existing matrices[11] or genetically manipulating mineralization of inorganic matrices[12].

I created a macroscopic, bottom-up, de novo ELM (BuD-ELM) by genetically engineering the freshwater bacterium Caulobacter crescentus[13] to display a surface bound synthetic matrix. For this purpose, I designed a bottom-up de novo (BuD) protein comprising three main domains (Fig. 1A): the N-terminal surface anchor of the native surface layer monomer (RsaA)[14], a central structural domain made of elastin-like polypeptides (ELPs) - a SpyTag[15] was also included for functionalization purposes, and the C-terminus of RsaA, necessary for extracellular secretion. In this way, I redesigned the bacterium surface (Fig. 1B), adding an external layer of synthetic proteins that self-associate due to the presence of the ELPs[16] and the highly hydrophobic RsaA Ctermini[17]. This strain was reported to form centimeter-scale material when grown in flasks. Through confocal microscopy, I observed that some of the BuD protein detached from the cell surface and formed a locally inhomogeneous extracellular matrix (Fig.1C), able to assemble cells into hierarchically-ordered macroscopic structures. I described the multi-step process of BuD-ELM formation that includes the early onset of a pellicle at the water-air interface that later sinks at the bottom of the flask (Fig.1D). Through genetic modifications of the matrix, I was able to control the stiffness of BuD-ELMs over a range that is ~50-fold greater than prior work (Fig.2A). I showed that BuD-ELMs can (1) generate new material even after being desiccated for up to three weeks (Fig.2B), (2) be used as water filters (Fig.2C), (3) functionalized with exogenous enzymes directly from complex mixtures (Fig.2D), (4) processed into a cohesive extrudable paste (Fig.3), and (5) form solid composites with inorganic powders (Fig.3).

This study offers a radically new design concept: that an extracellular matrix can mediate assembly of micrometer-sized cells into centimeter scale materials, thus organizing cells across four orders of magnitude. I anticipate the modularity of this approach will permit the incorporation of different protein polymers in the de novo matrix, allowing for tunable ELMs with a variety of desired structures and compositions of the bulk material. Moreover, combining specific matrix properties synergistically with existing cellular functions will accelerate development of biomaterials that grow, self-heal and regenerate, respond to environmental cues, reconfigure dynamically, and are multifunctional. Such biomaterials could sustain aging infrastructure with novel self-repairing structural materials and address sustainability challenges with synthesis under ambient conditions from dilute, low-purity starting materials. Thus, this work will enable a new era of biomaterials that would revolutionize our capabilities to meet the coming global challenges.

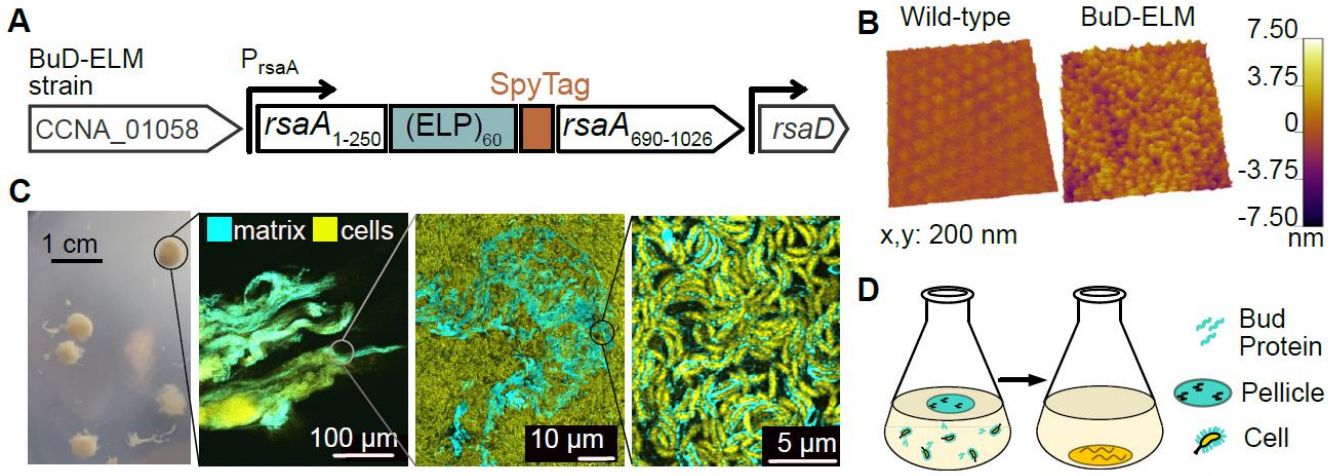

Figure 1: (A) Schematic of the construct replacing the native *rsaA* gene in the BuD-ELM strain of *C. crescentus*. **(B)** AFM images of the cell surface of wild-type (left), and BuD-ELM (right) strain. **(C)** Confocal microscopy of ELMs stained with SpyCatcher-GFP at increasing magnifications, showing a hierarchical structure. **(D)** Proposed mechanism for BuD-ELM formation.

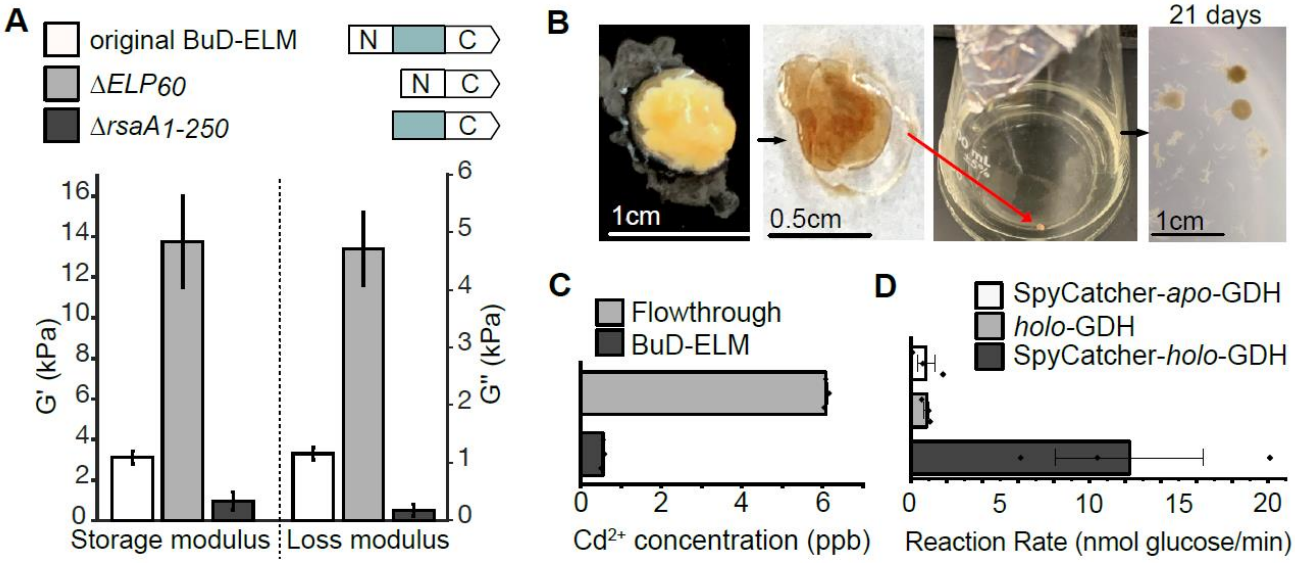

Figure 2: (A) Storage (G') and Loss (G'') modulus of original, *ΔELP*$_{60}$ and Δ*rsaA*$_{1\text{-}250}$ BUD-ELMs at an angular frequency of 10 rad s^{-1}. Error bars represent 95% confidence intervals. **(B)** Representative example of BuD-ELMs reseeding process, showing (from left to right): extraction from liquid culture, desiccated material, inoculation into fresh medium and formation of new BuD-ELM after 21 days. **(F)** Graph showing the final Cd^{2+} solution concentration after 6 ppb Cd^{2+} solution was incubated with (BuD-ELM) or without (flowthrough) the *ΔSpyTag* BUD-ELMs. Error bars represent standard error. **(G)** Graph showing the rate of glucose oxidation for BuD-ELMs that were incubated with SpyCatcher-*holo*-GDH, *holo*-GDH, or SpyCatcher-*apo*-GDH. Error bars represent standard error (GDH: oxidoreductase PQQ-glucose dehydrogenase).

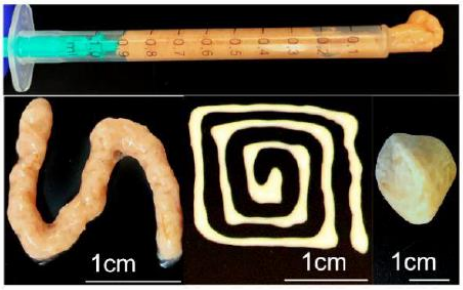

Figure 3: BuD-ELMs collected into a syringe (top) for extrusion using different-sized nozzles, (bottom-left and bottom-middle), showing their ability to be reshaped. BuD-ELMs mixed with glass powder to form a firm paste that hardens when dehydrated (bottom-right), showing its potential as cement-like agent.

References:

1. Wegst, U. G. K., Bai, H., Saiz, E., Tomsia, A. P. & Ritchie, R. O. Bioinspired structural materials. *Nature Mater* **14**, 23‑36 (2015).
2. Nguyen, P. Q., Courchesne, N. M. D., Duraj-Thatte, A., Praveschotinunt, P. & Joshi, N. S. Engineered Living Materials: Prospects and Challenges for Using Biological Systems to Direct the Assembly of Smart Materials. *Advanced Materials* **30**, 1704847‑1704847 (2018).
3. Srubar, W. V. Engineered Living Materials: Taxonomies and Emerging Trends. *Trends in Biotechnology* **39**, 574‑583 (2021).
4. Tang, T.-C. *et al.* Materials design by synthetic biology. *Nature Reviews Materials* 1‑19 (2020) doi:10.1038/s41578-020-00265-w.
5. Molinari, S., Tesoriero, R. F. & Ajo-Franklin, C. M. Bottom-up approaches to engineered living materials: Challenges and future directions. *Matter* **4**, 3095‑3120 (2021).
6. Wang, H. *et al.* Genetical Surface Display of Silicatein on Yarrowia lipolytica Confers Living and Renewable Biosilica‑Yeast Hybrid Materials. *ACS Omega* **5**, 7555‑7566 (2020).
7. Dorval Courchesne, N.-M., Duraj-Thatte, A., Tay, P. K. R., Nguyen, P. Q. & Joshi, N. S. Scalable Production of Genetically Engineered Nanofibrous Macroscopic Materials via Filtration. *ACS Biomater. Sci. Eng.* **3**, 733‑741 (2017).
8. Chen, A. Y. *et al.* Synthesis and patterning of tunable multiscale materials with engineered cells. *Nature Mater* **13**, 515‑523 (2014).
9. Chen, B. *et al.* Programmable living assembly of materials by bacterial adhesion. *Nat Chem Biol* 1‑6 (2021) doi:10.1038/s41589-021-00934-z.
10. Wang, Y. *et al.* Living materials fabricated via gradient mineralization of light-inducible biofilms. *Nat Chem Biol* **17**, 351‑359 (2021).
11. Gilbert, C. *et al.* Living materials with programmable functionalities grown from engineered microbial co-cultures. *Nature Materials* 1‑10 (2021) doi:10.1038/s41563-020-00857-5.
12. Kang, S.-Y. *et al.* Engineering Bacillus subtilis for the formation of a durable living biocomposite material. *Nat Commun* **12**, 7133 (2021).
13. Molinari, S. *et al.* A de novo matrix for macroscopic living materials from bacteria. 2021.11.12.468079 (2022) doi:10.1101/2021.11.12.468079.
14. Bharat, T. A. M. *et al.* Structure of the hexagonal surface layer on Caulobacter crescentus cells. *Nature Microbiology* **2**, 1‑6 (2017).
15. Zakeri, B. *et al.* Peptide tag forming a rapid covalent bond to a protein, through engineering a bacterial adhesin. *PNAS* **109**, E690‑E697 (2012).
16. Roberts, S., Dzuricky, M. & Chilkoti, A. Elastin-like polypeptides as models of intrinsically disordered proteins. *FEBS Letters* **589**, 2477‑2486 (2015).
17. Bingle, W. H., Nomellini, J. F. & Smit, J. Secretion of the Caulobacter crescentusS-Layer Protein: Further Localization of the C-Terminal Secretion Signal and Its Use for Secretion of Recombinant Proteins. *Journal of Bacteriology* **182**, 3298‑3301 (2000).