Synthetic Biology

FEI CAI



Current position: Researcher, Plant & Microbial Biology Department, University of California, Berkeley. Advisor: Dr. Cheryl A. Kerfeld.

Education: Ph.D. Biochemistry, Department of Chemistry and Biochemistry, The University of Southern Mississippi. Advisor: Dr. Gordon C. Cannon. B.S. Biology, Biology Department, Nanjing University, China.

Nonscientific interests: Traveling, badminton, cooking, and watching any animal behavior documentaries.

My current research interests involve the structure-based design and engineering of bacterial microcompartments and using modified microcompartment building blocks to produce proteinaceous architectures. This paper is a case of combining bioinformatics, structural biology and synthetic biology in bioengineering. I am also interested in developing synthetic biology tools in cyanobacteria, and believe it will enable the use of cyanobacteria for production of many valuable compounds. (Read Cai's article; DOI: 10.1021/sb500226j).

JAY H. CHOI



Jay H. Choi

Current position: Assistant Research Scientist, Johns Hopkins University. Advisor: Dr. Marc Ostermeier.

Education: Postdoctoral Fellow, Johns Hopkins University, Advisor: Dr. Marc Ostermeier; Ph.D. in Computational Biology,

University of California, San Francisco, Advisor: Dr. Fred E. Cohen; B.A/M.S. in Biochemistry/Computer Science, Washington University, Advisor: Dr. Gary Stormo.

Nonscientific interests: I enjoy traveling and love photography. I also enjoy winter sports such as skating, skiing, and snowboarding whenever possible.

My general research interest area is protein design and engineering. I am particularly interested in developing protein switches that can be potentially used for therapeutic and diagnostic purposes. Protein switches are engineered proteins that are composed of an input domain that recognizes and responds to an input signal and an output domain whose function is regulated by the state of the input domain. Engineered protein switches have a number of exemplary properties for sensing and therapeutic applications including a large dynamic range, high specificity for the activating ligand, and a modular architecture that will facilitate finetuning of the desired properties. Thus, protein switches that can be regulated through exogenous or endogenous inputs can have a broad range of biotechnological and biomedical applications. (Read Choi's article; DOI: 10.1021/ sb500254g).

JAN CLAESEN



Jan Claesen

Current position: Postdoctoral scholar, Department of Bioengineering and Therapeutic Sciences, University of California, San Francisco, CA, USA. Advisor: Dr. Michael A. Fischbach.

Education: Ph.D. in Biological Sciences, University of East Anglia, UK (2011). Advisor: Prof. Mervyn J. Bibb; M.Sc. (2006) and B.Sc. (2003) in Biological Engineering, KU Leuven, Belgium.

Nonscientific interests: Hiking, traveling, cooking, reading and music.

I am interested in the various functions that small molecule natural products fulfill for the bacteria that produce them. While these compounds can be developed as drugs that are administered via "classical" oral, topical or intravenous routes,

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their biosynthetic origin also allows for their targeted in situ delivery by engineered live bacterial cells. Several promising proof of concept studies have already highlighted the potential of bacterial cell therapy and are paving the path for development of self-contained bacterial "physicians", capable of disease diagnosis, drug administration as well as follow up. My future research will focus on the characterization of molecular mechanisms that mediate bacterial interspecies and microbe-host interactions in the human microbiome, as well as the application of these insights to the engineering of microbiota-derived diagnostics and therapeutics. (Read Claesen's article; DOI: 10.1021/ sb500258b).

ROGER R. DRAHEIM



Christine Oswald

Current position: Lecturer in Microbiology, Division of Pharmacy, Durham University, UK.

Education: Junior Research Group Leader (Institute of Biochemistry (Biozentrum), Goethe University Frankfurt, Germany); NIH Kirschstein NRSA Fellow (Prof Gunnar von Heijne, Stockholm University, Sweden); Ph.D. Microbiology (Prof. Michael D Manson, Texas A&M University, Texas, USA); B.S. Biochemistry and Microbiology, B.S. Molecular and Cellular Biology, University of Maine, Orono, Maine, USA.

Nonscientific interests: In my spare time, I enjoy cycling, traveling by backpack whenever possible, and mushroom hunting when the weather is good.

Two-component signaling circuits (TCSs) possess vast untapped engineering potential. They govern the majority of environmental, pathogenic and industrial processes undertaken by bacteria. Therefore, controlling signal output from these circuits in a stimulus-independent manner is of central importance to synthetic microbiologists. Aromatic tuning, or repositioning the aromatic residues commonly found at the cytoplasmic end of the final transmembrane helix has been shown to modulate signal output from several TCSs. We are currently designing synthetic bacterial signaling pathways based on aromatic tuning in order to develop a next-generation biological platform for high-throughput detection of compounds with novel antimicrobial activity. In addition, we have recently expanded into engineering of metabolite biosensors composed of aromatically tuned two-component systems that are amenable to adaptive laboratory evolution. (Read Draheim's article; DOI: 10.1021/sb500261t).

Introducing Our Authors

LAUREN E. S. GULLAND



Stuart Gulland

Current position: Senior in high school, graduating in June 2015; currently eagerly awaiting college admission decisions!

Education: Student at Mira Costa High School in Manhattan Beach, California. Research Advisors: Dr. Avena Ross and Professor Bradley Moore at the Scripps Institution of Oceanography, UCSD. I'd also like to thank Dr. Komives and the UCSD Academic Connections Program for giving me such an incredible opportunity.

Nonscientific interests: I enjoy playing the euphonium and trombone in our school's wind ensemble and marching band, and am a co-captain of both our school Science Olympiad team and our local FIRST Robotics team. In my free time, I've recently started learning Korean.

I plan on studying biomechanical engineering in the future; working in Professor Moore's lab has been an amazing experience that goes far beyond any typical high school classroom. Through this process, I've solidified my interest in wanting to dive into research; I loved everything that we did in the lab, and learned an insane amount throughout the course of my time there. The entire Moore group was really encouraging and stimulating, and Dr. Ross, my mentor, let me take over her research almost completely for the duration of my stay, teaching me everything from lab discipline and procedures to university-level biochemistry concepts. I look forward to having more research experiences at the university level, and cannot wait to be a full-time college student. (Read Gulland's article; DOI: 10.1021/sb500280q).

YOSHIKI HIGUCHI



Current position: Professor, Department of Picobiology, Graduate School of Life Science, University of Hyogo, Hyogo, Japan.

Education: Doctor of Science (1984), B.S. Polymer Science (1979), Osaka University, Advisor: Prof. Masao Kakudo.

Nonscientific interests: I enjoy gardening, cleaning rooms and repairing furniture at home.

I have been interested in the structure-function relationship of proteins. My current research is focused on X-ray crystallographic studies of (1) hydrogenases, (2) protein function in the Wnt signaling system, and (3) proteins that are useful for green chemistry. This paper shows protein engineering of L-Proline cis-4-hydroxylases (cis-P4Hs) to facilitate the conversion of the majority of L-Pipecolic acid (L-Pip) into the cis-5-hydroxy-L-pip (cis-5-Hypip) isomer. cis-P4Hs from Sinorhizobium meliloti and Mesorhizobium loti catalyze the hydroxylation of L-Proline, generating *cis*-4-hydroxy-L-proline, as well as the hydroxylation of L-Pip, generating two regioisomers, cis-5-Hypip and cis-3-Hypip. To selectively produce *cis*-5-Hypip without simultaneous production of two isomers, protein engineering of cis-P4Hs has been carried out based on the X-ray crystal structure of cis-P4H in complex with each of L-Proline and L-Pip. (Read Higuchi's article; DOI: 10.1021/sb500247a).

YUKIKO KAMIYA



Yukiko Kamiya

Current position: Lecturer, EcoTopia Science Institute, Nagoya University, Japan.

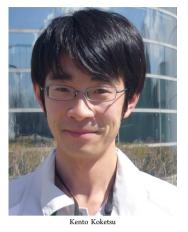
Education: Ph.D., Graduate School of Pharmaceutical Sciences, Nagoya City University, Japan. Advisor: Prof. Koichi Kato; Postdoctoral Fellow, Institute for Molecular Science, National Institutes of Natural Sciences, Japan.

Nonscientific interests: I enjoy traveling, hiking in nature, music.

My research interest is the design of molecular tools that can be applied to biological systems. In particular, I am focusing on creating oligonucleotide analogues. Modification with azobenzene, a photochromic molecule, into DNA allows us to control hybridization of DNA and interaction with protein. In this paper we demonstrated the photoregulation of gene expression by controlling the transcription reaction mediated by interaction between DNA and polymerase. I believe that this unique tool can be used as a regulatory system to investigate the dynamic ordering of protein complexes embodying biological properties and to construct the artificial cells. I am also interested in the endogenous gene regulation system, RNA interference, and have designed RNA-based molecular tools for investigating how RNAi works in cells. (Read Kamiya's article; DOI: 10.1021/ sb5001092).

Introducing Our Authors

KENTO KOKETSU



Current position: Researcher, Bioprocess Development Center, Kyowa Hakko Bio Co., Ltd.

Education: Ph.D. Chemistry (2009), B.S. (2005), Hokkaido University. Advisor: Prof. Hideaki Oikawa.

Nonscientific interests: I enjoy traveling with my family and playing football.

I am interested in the microbial production of useful materials such as nonproteinogenic amino acids. In this report, we achieved the microbial hydroxylation of L-Pipecolic acid using Escherichia coli expressing L-proline-cis-4-hydroxylase in a regioand stereoselective manner. Initially, hydroxylation of L-pipecolic acid using the wild type enzyme showed low selectivity, but the protein engineering based on the crystal structure with the substrates, L-pipecolic acid and α -ketoglutarate, allowed the selective production of the desired *cis*-5-hydroxypipecolic acid. Throughout this research, I learned that the protein engineering is powerful tool to develop the novel bioprocess generating valuable materials. I also have an interest in the biosynthesis of secondary metabolites, particularly, unusual enzymatic reactions. In the future I hope to develop bioprocesses using unusual enzymes. (Read Koketsu's article; DOI: 10.1021/sb500247a).

CARLOS PIÑERO LAMBEA



Carlos Piñero Lambe

Current position: Ph.D. candidate at Centro Nacional de Biotecnología (CNB-CSIC), Madrid, Spain. Advisors: Drs. Luis Ángel Fernández and Gustavo Bodelón.

Education: M.Sc. Biotechnology, B.Sc. Biochemistry, Universidad Autónoma de Madrid (Spain).

Nonscientific interests: Running, soccer, skiing, spending time with family and friends.

My research is focused on engineering bacteria for biotechnological applications. In this work we designed and constructed synthetic adhesins (SAs) that direct the specific attachment of Escherichia coli to different target surfaces, cells and tumors. SAs are based on the smallest recombinant antibody fragments known to date, the so-called VHHs, which can be selected from large repertoires, enabling the programming of bacterial adhesion to virtually any antigen of interest. We show that constitutive expression of SAs from the chromosome is stably maintained without perturbing bacterial growth. Importantly, SAs allow bacteria to efficiently colonize target solid tumors using low doses of administered bacteria. I am convinced that SAs will help to expand the use of bacteria as living therapeutics (*i.e.*, vaccines, vehicles for drug release) and industrial components (*i.e.*, biosensors, bioreactors). (Read Lambea's article; DOI: 10.1021/ sb500252a).

YI LIU



Current position: M.D./Ph.D. Candidate, Interdisciplinary Biological Sciences, Northwestern University. Advisor: Dr. Michael C. Jewett.

Education: B.A. Chemical and Physical Biology, Harvard University. Advisor: Dr. David Jeruzalmi.

Nonscientific interests: Women's health advocacy, intersectional feminism, applied arts.

My current research interests are primarily focused on better understanding the translational tolerance and fidelity of the E. coli 70S ribosome at the molecular level. My approach is to engineer synthetic ribosomes in vitro to assess the mutational space possible for ribosome construction and evolution specific to targeted peptide synthesis. Such efforts have previously been hampered by limitations within the in vitro ribosome synthesis, assembly, and translation (iSAT) system, including short reaction duration, diminished yields, energy exhaustion, and toxic byproduct accumulation. Here, we quantified substrate limitations to be dependent on diminishing adenylate energy charge over time, as well as inorganic phosphate accumulation, which inhibits translation. To alleviate these limitations, we employed a semicontinuous format which increased iSAT reaction duration 5-fold and protein synthesis yields 7-fold. My work will aid efforts to build minimal cells and open new avenues for making ribosomes with altered properties. (Read Liu's article; DOI: 10.1021/ sb5002467).

Introducing Our Authors

ARNAB MUKHERJEE



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Current position: James G. Boswell Postdoctoral Fellow in Molecular Engineering & Magnetic Resonance Imaging, California Institute of Technology.

Education: Postdoctoral Fellow, California Institute of Technology, Advisor: Prof. Mikhail G. Shapiro; B.Tech and M.Tech, Indian Institute of Technology (IIT), Madras, Advisors: Prof G.K. Suraishkumar (IIT, Madras) & Prof. Peter Uetz (Virginia Commonwealth University).

Nonscientific interests: I enjoy reading and occasionally writing poetry and watching noir and "zombie"-themed movie classics.

My research interest focuses on repurposing proteins to develop genetically encoded probes for biological imaging. In particular, I am interested in developing new reporters to enable fluorescence imaging in low-oxygen conditions, which are typically encountered in several clinically and industrially relevant biosystems (e.g., microbial fermentation, rumen microbiota, and biofilms). Furthermore, I am interested in leveraging the fluorescent reporters described in our work in ACS Synthetic Biology to develop a new class of fluorescence-based O₂independent sensors for biomolecules such as ATP and calcium. In addition, I am keenly interested in clinically relevant imaging modalities such as magnetic resonance imaging (MRI). To this end, I am currently engaged in repurposing metalloproteins to develop genetically encoded dynamic contrast agents for noninvasive detection of biomolecules (e.g., ATP, cAMP) using MRI. (Read Mukherjee's article; DOI: 10.1021/sb500237x).

WEI NIU



Jiantao Guo

Current position: Research Assistant Professor, Department of Chemistry, University of Nebraska–Lincoln.

Education: Ph.D. Chemistry, Michigan State University. Advisor: John W. Frost; B.S. Chemistry, Tsinghua University, Beijing, China.

Nonscientific interests: I enjoy all types of outdoor activities, ranging from hiking to gardening.

My research interests focus on building microbial cell factories for the purpose of synthesizing value-added small molecules and macromolecules. These are multifaceted challenges, which quite often have biosynthetic pathway engineering at the center of the quests. In this paper, we questioned the conventional practice of direct-applying naturally existing pathways in the biosynthesis of a small-molecule natural products, by designing an artificial biosynthetic pathway of 1,2-propanediol that overcomes inherent drawbacks associated with the natural biosynthesis. The devised pathway hijacked the mixed acid-fermentation of *E. coli* through the reduction of lactic acid to the target molecule. We successfully demonstrated the feasibility of the reduction steps of our pathway together with its unique feature as a stereospecific synthesis for each 1,2propanediol enantiomers. (Read Niu's article; DOI: 10.1021/ sb500240p).

AVENA C. ROSS



Matthew Zamora

Current position: Queen's National Scholar and Assistant Professor at Queen's University, Kingston, Canada.

Education: Postdoctoral Fellow, UCSD, with Bradley S. Moore (2012–2014); Ph.D. Chemistry University of Alberta, with John C. Vederas (2012); B.Sc. (Hons) Chemistry at the University of Auckland (2006).

Nonscientific interests: I enjoy spending time with friends, sharing good food and wine. I also love chamber music and hiking in the great outdoors!

I am fascinated by how nature assembles natural products, especially the elegant shortcuts and tricks employed to produce a complex molecule from basic building blocks. Using genetic approaches, such as our Transformation Associated Recombination (TAR) platform, we can now capture and study any biosynthetic pathway with accompanying sequence information. The full scope of bacterial secondary metabolism, therefore, becomes accessible and by interrogation of silent and hard to access pathways, such as those in uncultured organisms, we have the opportunity to uncover pathways and molecules that were previously unattainable. Research in my group currently focuses on using innovative approaches such as TAR to uncover and study the biosynthetic mechanisms for molecules and pathways from marine proteobacteria, particularly noncanonical and silent pathways from the genus Pseudoalteromonas. (Read Ross' article; DOI: 10.1021/sb500280q).

JENNIFER A. SCHOBORG



Saoirse McSharry

Current position: Ph.D. candidate, Department of Chemical & Biological Engineering, Northwestern University. Advisor: Prof. Michael C. Jewett.

Education: B.S., Department of Chemical and Biological Engineering, Iowa State University. Advisors: Prof. Derrick K. Rollins and Prof. Balaji Narasimhan.

Nonscientific interests: Playing intramural sports, singing in a graduate student a cappella group.

My research interests center around protein production and design, particularly for pharmaceuticals. The exciting challenge of producing proteins that are difficult to express, as well as entire molecular machines like the ribosome, led me to study cell-free systems. My work primarily lies in the use of yeast and E. coli cell-free protein synthesis systems, where I've learned about the conditions needed to produce a variety of challenging proteins including human granulocyte macrophage colony stimulating factor and erythropoietin. In addition to research, I'm passionate about engineering education and had the opportunity to coteach undergraduate fluid mechanics in a Teaching Apprenticeship Program. Looking forward for the featured research, the iSAT system progress described can allow for substantial quantities of designer ribosomes capable of making novel molecules that were previously impossible with in vivo techniques. (Read Schoborg's article; DOI: 10.1021/ sb5002467).

YASUHITO SHOMURA



Yasuhito Shomura

Current position: Assistant Professor, Graduate School of Life Science, University of Hyogo (Associate Professor, Graduate School of Science and Technology, Ibaraki University, from April 2015).

Education: Ph.D. (Science), Kyoto University (2003). Advisor: Dr. Kunio Miki.

Nonscientific interests: Reading and drinking.

One of my current interests concerns structure-based protein engineering that modifies and optimizes the substrate specificity of the target enzymes. In this paper we have succeeded in the X-ray structure determination of the L-proline *cis*-4-hydroxylase in full complex with the cofactors and substrate. The structures have unambiguously revealed the interaction modes between the enzyme and substrates at the atomic level. Unfortunately for us, however, we have failed to determine how the random mutation affected the differences in the regio- and stereoselectivity of the reaction. In the future, I hope to develop a theoretical approach to give convincing interpretations between the structural differences and substrate/product specificities, which would be powerful tool for the rational design of industrially beneficial enzymes. (Read Shomura's article; DOI: 10.1021/sb500247a).

MARKUS SUTTER



Markus Sutter

Current position: Senior Research Associate, Lawrence Berkeley National Lab, Berkeley, CA and MSU-DOE Plant Research Laboratory, East Lansing, MI.

Education: Ph.D., ETH Zurich, Switzerland, Advisors: Prof. N. Ban and E. Weber-Ban, Undergraduate/Graduate: ETH Zurich, Switzerland.

Nonscientific interests: Photography, climbing, hiking.

My research focuses on bacterial microcompartments, which are subcellular proteinaceous organelles designed to contain reactions that need to be separated from the cytosol. I find them fascinating because researching them encompasses so many aspects of proteins. There are many questions to be answered on how they evolved, the way they are assembled, how substrates and products get into and out of the shell, and what happens to them when they are not needed anymore. (Read Sutter's article; DOI: 10.1021/sb500226j).

LENNART SCHADA VON BORZYSKOWSKI



Lennart Schada von Borzyskowski

Current position: Ph.D. Candidate, ETH Zürich, Switzerland & Max-Planck-Institute for Terrestrial Microbiology, Marburg, Germany; Advisor: Dr. Tobias J. Erb.

Education: M.Sc. in Applied Natural Sciences, Technical University Freiberg, Germany; Visiting Scholar at the California Institute of Technology, USA; Advisor: Prof. André Hoelz.

Nonscientific interests: I enjoy traveling to discover new countries and learn new languages, listening to live music and reading about history.

My research is focused on developing new genetic tools for Alphaproteobacteria. This class comprises species with a broad metabolic diversity, including both autotrophs and heterotrophs, and offers numerous unique opportunities for biological engineering. With my publication, I want to increase the possibilities to genetically manipulate Alphaproteobacteria and harness their potential for synthetic biology. Using fluorescent reporter proteins and single-cell microscopy, I could show that newly developed expression systems are functional in several alphaproteobacterial model species, and demonstrate that a short pathway is expressed functionally as a proof of principle. Continuing my research, I aim at developing additional sophisticated genetic tools and engineering Alphaproteobacteria for the production of value-added compounds from low-cost substrates, e.g., methanol. (Read von Borzyskowski's article; DOI: 10.1021/sb500221v).