# Synthetic Biology-

# FARHIMA AKTER



Farhima Akter

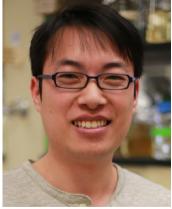
**Current Position.** Postdoctoral researcher, Department of Biomedical Engineering, University of California, Davis, USA. Advisor: Dr. Yohei Yokobayashi.

**Education.** M.S. and Ph.D. Biological Information, Tokyo Institute of Technology, Japan (2012). Advisor: Dr. Eiry Kobatake; B.Sc. Microbiology, University of Dhaka, Bangladesh (2005).

**Nonscientific Interests.** Traveling, spending time with family and friends.

My general research interest area is engineering of macromolecules (protein and nucleic acid) for biosensing. In my Ph.D., I was particularly interested in developing fusion proteins that can be potentially used for development of DNA based immunoassays in diagnostic purposes. This paper is a case of RNA engineering that is involved in an *in vitro* RNA circuit capable of signal amplification; an input RNA strand catalytically activates multiple Spinach aptamers via nonenzymatic RNA strand displacement reactions. My current research interests involve the structure-based design, engineering and characterization of luciferase for activable MRI probes. (Read Akter's article; DOI: 10.1021/sb500314r).

## ZEHUA BAO



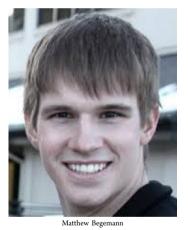
Xiong Xiong

**Current Position.** Ph.D. Candidate, Department of Biochemistry, University of Illinois at Urbana–Champaign, Urbana, IL. Advisor: Prof. Huimin Zhao. **Education.** B.S. in Biological Sciences and Biotechnology, School of Life Sciences, Tsinghua University, Beijing, China. Advisor: Prof. Zihe Rao.

**Nonscientific Interests.** Basketball, tennis, table tennis, billiards, outdoors, photography, reading, Chinese calligraphy.

My research interests lie in engineering genomes and transcriptomes in an efficient and controlled manner. Genome engineering and gene regulation tools play a fundamental role in understanding biology, engineering metabolism and developing therapies. However, current tools to facilitate such applications are inefficient and lowthroughput, often hindering our ability to explore beyond the technical limit. In this paper, we utilized the innate multiplicity of CRISPR/Cas9 system to modify the yeast genome in a multiplexed and efficient manner. This could facilitate the creation of designer yeast strains that can be used in directed protein evolution or metabolic engineering. Looking forward, I am continuing to improve the multiplicity as well as reliability of our system. I am also developing a continuous genome evolution strategy in yeast and a ligandcontrolled gene regulation system in mammalian cells. (Read Bao's article; DOI: 10.1021/sb500255k).

#### MATTHEW BEGEMANN



Current Position. Associate Scientist, Benson Hill Biosystems, St. Louis, Missouri.

Education. Ph.D. Microbiology, University of Wisconsin, Madison (2014). Advisor: Brian Pfleger. B.S. Biochemistry, University of Missouri, Columbia (2009). Advisor: Judy Wall.

**Nonscientific Interests.** Hunting, fishing, walking my hound dog, and home brewing beer.

I am interested in the application of synthetic biology to improve photosynthesis. I am currently working on developing traits to improve photosynthesis in crop plants, particularly under abiotic stress. Photosynthesis is a challenging topic due to

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the structural, biochemical, and gene-regulatory complexity of the system. Research on photosynthetic organisms, both plants and microorganisms, is especially difficult due to a shortage of defined synthetic biology parts and tools. Development of new enabling technologies, such as the gene expression tools characterized in our recent paper, will be essential to the future application of photosynthetic organisms for the production of food, fuel, and fiber. (Read Begemann's article; DOI: 10.1021/ sb500260k).

# LARA BEREZA-MALCOLM





Current Position. Ph.D. Candidate, Department of Physiology, Anatomy and Microbiology, School of Life Sciences, College of Science, Health and Engineering, La Trobe University, Melbourne, Victoria, Australia. Advisor: Dr. Ashley Franks and Dr. Gülay Mann.

Education. B.Sc. (Hons first class) Microbiology, La Trobe University.

Nonscientific Interests. Exploring new countries, hiking and watching movies, particularly sci-fi and horror.

Our paper explores whole cell microbial biosensors, which have been developed for the detection of heavy metals. Microbial biosensors previously designed have been focused on modular, promoter/reporter gene pathways, allowing quantitative and qualitative analysis of contaminated environments. Further development is resulting in multiplexed biosensing systems, to allow for increased biosensor flexibility. The aim of microbial biosensor development is for real-world application, allowing at risk communities rapid, and specific, on-site detection of potentially contaminated soils and waterways. There are, however, multiple difficulties hindering this movement, including the toxic nature of heavy metals. Thus, we propose a standard of criteria to be applied to future biosensor pathway design. This framework will allow accelerated movement from laboratory-based designs, to those ready for application. We also examined potential risks and regulations associated with development of genetic modified microbes to be used with environmental samples. Currently, our lab is working on the development of a range of heavy metal biosensors to be applied for long-term detection in at-risk environments. (Read Bereza-Malcolm's article; DOI: 10.1021/sb500286r).

#### JAMES CHAPPELL



James Chappell

Current Position. Postdoctoral Researcher, Department of Chemical and Biomolecular Engineering, Cornell University, Ithaca, NY. Advisor: Prof. Julius B Lucks.

Education. Ph.D. Molecular Bioscience, Imperial College London, UK. Advisor: Prof. Paul Freemont. B.S. Biochemistry, Imperial College London.

Nonscientific Interests. I enjoy traveling, sports and music.

My general research interests are in understanding and engineering regulatory mechanisms to precisely control gene expression. In particular, I am interested in engineering small RNA regulators of transcription for this purpose. In this article, we describe how in vitro transcription and translation (TX-TL) reactions can be applied to rapidly characterize and prototype RNA circuitry. Harnessing such in vitro reactions promises to speed up current "design-build-test" cycles for more efficient engineering of biological systems. (Read Chappell's article; DOI: 10.1021/sb400206c).

# RYAN CLARKE



Ryan Clarke

Current Position. First year Ph.D. Student, Department of Biochemistry and Molecular Genetics, University of Illinois-Chicago. Advisor: Dr. Bradley Merrill.

Education. Biochemistry, University of Wisconsin-Madison. Advisor: Prof. Brian Pfleger.

Nonscientific Interests. Aside from traveling outside of the USA and watching sci-fi flicks, I spend my free time working on transforming new ideas into businesses and working with startups in general. I enjoy trying to apply the scientific problem solving mindset to entrepreneurial processes.

My lab is interested in the optimization and further development of the CRISPR-cas9 genome engineering system for general use in mammalian cells, and more specifically embryonic stem cells. We are particularly interested in what keeps stem cells in their most robust pluripotent state, the naïve state, and what drives them to differentiate. My research will surround the usage of novel applications of the cas9 system for further characterization of naïve pluripotent stem cells to continue to lay the framework for translational stem cell studies and applications. (Read Clarke's article; DOI: 10.1021/sb500260k).

## WOUTER ENGELEN



Bart van Overbeeke

**Current Position.** Ph.D. candidate, Department of Biomedical Engineering, Eindhoven University of Technology, Netherlands; Advisor: Dr. M. Merkx.

**Education.** M.Sc. Biomedical Engineering, Eindhoven University of Technology, Netherlands.

Nonscientific Interests. Traveling and music.

My general interest lies in the design and development of synthetic molecular systems inspired by processes observed in cells. Using the programmable nature of Watson–Crick base pairing, DNA has proven to be a versatile building block for these molecular circuits. This paper presents the DNA-templated, reversible assembly of an enzyme–inhibitor pair. By directing enzyme activity using various DNA triggers, the developed switch provides an interesting read-out probe for DNA-based molecular circuits. In addition, interfacing DNA-circuits with enzyme activity might make it possible to create complex networks of DNA-circuits interacting with cascades of multiple enzymes. (Read Engelen's article; DOI: 10.1021/sb500278z).

# ASHLEY FRANKS



Tess Flynn

**Current Position.** Senior Lecturer and Head of Applied Environmental Microbiology Laboratory, Department of Physiology, Anatomy and Microbiology, School of Life Sciences, College of Science, Health and Engineering, La Trobe University, Melbourne, Victoria, Australia.

**Education.** Ph.D. Microbiology, University of California, University of New South Wales, Australia (2005). Advisor: Prof Staffan Kjelleberg; B.Sc. Microbiology (Hons first class), University of New South Wales, Australia (1999).

**Nonscientific Interests.** Train travel especially by steam engine, food, cooking, culture and history. I usually like to try and combine these while traveling.

My laboratory is interested in investigating natural systems with potential applied applications. Our research combines microbial ecology, bioremediation, electromicrobiology, and microbiome research. Through understanding microbial processes within the environment, and their drivers that select microbial diversity and function, we have the possibility to provide beneficial outcomes to real world problems. Synthetic biology is rapidly developing a framework to apply microbes to work for us in a systemic manner. To fully achieve the potential that bacteria offer, such as acting as biosensors, we need to define the requirements for our needs. Questions such as sensitivity and specificity requirements in real world settings need to be considered. By providing a framework for whole cell microbial biosensor development, research programs can be standardized for a common benefit to all. (Read Franks' article; DOI: 10.1021/ sb500286r).

#### ANDREW DAVID GARST



Andrew David Garst

**Current Position.** Postdoctoral research associate, University of Colorado Boulder.

**Education.** Ph.D. in Biochemistry, University of Colorado Boulder; Bachelors of Science in Biology, New Mexico Highlands University.

**Nonscientific Interests.** In my free time I enjoy binging on Netflix, hiking, biking, playing the piano, goofing around with my kids and reading science fiction and fantasy novels.

Generally speaking I am interested in developing technologies that enable rapid and efficient genome scale engineering. The codon compression algorithms that were developed in this work are part of this larger goal as they are aimed at providing maximal control over the codon composition of degenerate DNA oligonucleotides that would be used to generate genomic libraries. These algorithms allow control over amino acid composition and can take into account organism specific codon usage patterns. Instead of providing a general

solution to such a problem, this work aimed to find ways to specifically tailor the degeneracy based on the desired amino acids and organism while still minimizing the number of oligonucleotides required. Such approaches to library design should enable more economic ways to generate sophisticated libraries by significantly decreasing the oligo/library ratio and minimizing the high the screening loads typically associated with random mutagenesis. It is our long-term goal to incorporate these concepts into synthetic library designs for genome engineering efforts and scale such approaches to parallel oligo synthesis platforms. (Read Garst's article; DOI: 10.1021/sb500282v).

# BRIAN JANSSEN



**Current Position.** Ph.D. Candidate in Chemical Biology, Department of Biomedical Engineering, Eindhoven University of Technology (TU/e), Eindhoven, The Netherlands, Advisor: Dr. M. Merkx.

Education. Masters in Biomedical Engineering (TU/e), The Netherlands, Advisors: Dr. M. Merkx and Prof. Dr. B. Meijer; Internship: Wyss Institute/Harvard Medical School/ Dana-Farber Cancer Institute. Boston, Massachusetts (USA), Advisors: Dr. W. Shih, Dr. C. Lin and Dr. F. Graf.

Nonscientific Interests. Football, squash and guitar.

Deoxyribonucleic acids, or DNA, store the blueprint for life, but the highly predictable Watson-Crick base pairing also makes DNA an attractive material for bottom-up nanotechnology. In the past decade, nucleic acids were proven to be promising building blocks to develop molecular nanostructures and dynamic nanodevices that may find applications in biomedicine and molecular diagnostics. An important challenge in this field is to develop generic approaches that interface DNA based logics with protein activity. My Ph.D. focused on novel biomolecular approaches to use DNA to control protein-based interactions, in particular to reversible control the activity of enzymes and antibodies. This paper represents a modular approach to couple DNA-based reactions with enzyme activity that could be used for DNA-detection purposes as well as readout for DNA-logic operations. (Read Janssen's article; DOI: 10.1021/sb500278z).

Introducing Our Authors

# JOSÉ JIMÉNEZ



José Jiménez

**Current Position.** Lecturer in Synthetic Biology, Faculty of Health and Medical Sciences, University of Surrey (United Kingdom).

Education. Postdoctoral Fellow at MIT (advisor: Domitilla Del Vecchio), Harvard University (advisor: Irene Chen) and CNB-CSIC (Spain, advisor: Victor de Lorenzo). Ph.D. Biochemistry and Molecular Biology (advisors: Eduardo Diaz and Jose Luis Garcia) and B.S. Biochemistry. Universidad Complutense de Madrid (Spain).

**Nonscientific Interests.** I used to like watching good series and any sport outdoors, especially surfing and cycling. I also used to be a terrible rock drummer. Now I am a full time (and happy) dad of twins.

I am interested in learning about fundamental properties of biological systems through building artificial genetic systems. More specifically, I am currently working in the quantitative understanding of the interplay between genetic circuits and their host as well as in the evolutionary optimization of this interaction. Applications inspired by other engineering disciplines, such as the insulator described in the paper, help to mitigate the burden generated by the production of exogenous genes in the cell, and also allow to understand the role of certain network motifs, like futile cycles, in nature. (Read Jiménez's article; DOI: 10.1021/sb5002533).

## SUNG IN LIM



Sung In Lim

**Current Position.** Ph.D. candidate in Chemical Engineering, University of Virginia. Advisor: Dr. Inchan Kwon.

Education. M.S. in Chemical Engineering, Pohang University of Science and Technology (POSTECH), South Korea.

Advisor: Dr. Gyoo Yeol Jung; B.S. in Chemical and Biological Engineering, Seoul National University, South Korea.

Nonscientific Interests. I enjoy watching SF movies and playing soccer.

My research interest is to develop new biologics and biobetters. In particular, I focus on targeted drug delivery, half-life extension, and multifunctional drug conjugate by employing protein evolution and bioconjugation. For example, therapeutic proteins are site-specifically attached through bioorthogonal chemistry to any functional molecules such as cytotoxic agents, PEGs, and aptamers to yield biotherapeutic conjugates with improved potency, extended half-life, and targeting efficiency. My current study concerns development of (1) a long-acting therapeutic enzyme by sitespecific albumination, (2) methods for subcellular delivery of therapeutic proteins, and (3) protein immobilization techniques for diagnostic purposes. (Read Lim's article; DOI: 10.1021/sb500309r).

# GÜLAY MANN



Tess Flynn

**Current Position.** Principal Research Scientist (Synthetic Biology), Chemical and Biological Defense Branch, Land Division, Defense Science and Technology Organisation, Melbourne, Victoria, Australia.

Education. Ph.D. Cereal Chemistry, Biochemistry and Rheology, University of Reading, United Kingdom (2001). Advisor: Prof J. David Schofield; B.Sc. Food Science (Hons first Class), University of Reading, United Kingdom (1998).

**Nonscientific Interests.** Traveling and exploring different cultures, learning languages, dress making, healthy food and bushwalking.

I have been developing a collaborative research program in Synthetic Biology as one of the emerging (bio)technologies of the future that will have a significant impact on the global health, safety and economies. While we have started our research on developing microbial biosensors for the detection of heavy metals, the ultimate objective of our research program is to develop microbial biosensors that can detect and degrade pollutants in remote environments or the sensing of explosives and biological-warfare agents. (Read Mann's article; DOI: 10.1021/sb500286r).

#### ANDREW MARKLEY



Arthi Padmanabhan

Current Position. NSF Postdoctoral Fellow, University of Wisconsin-Madison, Advisor: Brian Pfleger.

**Education.** Ph.D. Biochemistry, University of California, San Diego, Advisor: Jack Dixon; B.S. Chemistry, Carnegie Mellon University.

**Nonscientific Interests.** I enjoy competing in a variety of team sports, especially Ultimate Frisbee. I am also an avid gardener, homebrewer and photographer.

My doctoral work focused on a family of ribosomally encoded natural products. My current professional interests are focused on addressing intractable ecological problems with new biological systems. The marine cyanobacteria, *Synechococcus* sp. strain PCC 7002, has the potential to be an economically viable production strain for these efforts. However, we quickly found that better genetic control was needed for efficient engineering of this organism. The induction systems and promoter libraries described in this paper have proven very useful in our lab's ongoing engineering efforts. (Read Markley's article; DOI: 10.1021/sb500260k).

## KAYZAD SOLI NILGIRIWALA



Kayzad Soli Nilgiriwala

**Current Position.** Research Officer, The Foundation for Medical Research, Mumbai, India.

Education. Postdoctoral Research Associate (2010–2013), Department of Mechanical Engineering, Massachusetts Institute of Technology (MIT), Cambridge; Advisor: Prof. Domitilla Del Vecchio; Ph.D. in Microbiology (2003–2009), Mumbai University, Mumbai, India; Advisor: Prof. Shree Kumar Apte, Bhabha Atomic Research Centre (BARC), Mumbai, India.

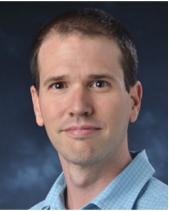
#### ANALISE Z. REEVES

Nonscientific Interests. Swimming, badminton, table tennis, and chess.

I am currently studying multidrug resistant (MDR) and extensively drug resistant (XDR) clinical bacterial strains of *Mycobacterium tuberculosis* toward understanding the mechanisms of phenotypic and genotypic drug resistance. My doctoral research involved cloning of a novel alkaline phosphatase from a *Sphingomonas* sp. toward studying its biochemistry and standardizing its application in bioprecipitation of heavy metals from alkaline solutions. In my postdoctoral research at MIT, we constructed one of the first biomolecular insulator circuits *in vivo* in *Escherichia coli* in order to study the effects of retroactivity and insulation in a two-component signal transduction system.

In the paper described, we demonstrate that the input/output gain of a transcriptional unit (activated by phosphorylation) can be tuned by altering the relative amounts of the substrate (transcription factor) and its cognate phosphatase by using an amplifying buffer circuit with tunable gain. (Read Nilgiriwala's article; DOI: 10.1021/sb5002533).

## **GUR PINES**



Gur Pines

**Current Position.** Postdoctoral scholar, Department of Chemical and Biological Engineering, University of Colorado, Boulder, CO, USA. Advisor: Prof. Ryan T. Gill.

**Education.** Ph.D. and M.Sc. in Life Sciences, the Weizmann Institute of Science, Israel. Advisor: Prof. Yosef Yarden. B.Sc. in Animal Sciences, the Hebrew University of Jerusalem, Israel. Advisor: Prof. Rina Meidan.

**Nonscientific Interests.** Hiking and rock-climbing. Recently I started enjoying the Colorado ski slopes. I also collect old science books and appreciate how far we progressed.

My graduate studies focused on cancer research, during which I was exposed to the field of Synthetic Biology. Currently I am involved in developing tools for genome engineering in bacteria, which includes harnessing the power of evolution rather than fighting against it (as in cancer). The current paper started as a thought experiment I had with my brother Assaf Pines, as means to reduce library size in saturation mutagenesis using degenerate nucleotides. Together with other lab members, we came up with a computational tool that allows us to compress codons. This was also demonstrated experimentally using the genomic engineering tools we develop in the lab. In the future, I hope to combine the knowledge I gained in both fields and develop tools for bioengineering of both prokaryotic and eukaryotic genomes. (Read Pines' article; DOI: 10.1021/sb500282v).



Scott Goldstein

**Current Position.** Postdoctoral fellow, Department of Medicine (Infectious Diseases), Massachusetts General Hospital, Harvard School of Medicine, Boston, MA. Advisor: Dr. Cammie F. Lesser.

**Education.** Ph.D. Microbiology and Molecular Genetics, Emory University, Atlanta, GA (2010). Advisor: Dr. Thomas Shinnick; B.S. Microbiology, University of Louisiana, Lafayette, LA (2004). Advisor: Dr. Don Ennis.

**Nonscientific Interests.** Outside of the lab, I am an avid runner and triathlete who loves to travel and cook. I also teach cycling classes at fitness centers around Boston, MA and compete as a member of the USA Triathlon Team.

My current research is focused on designing and developing bacteria-based therapeutics by engineering and repurposing intrinsic tools possessed by microbes. I am particularly interested in type 3 secretion systems, which are nanomachines that can be engineered to deliver proteins directly into the cytoplasm of mammalian cells or into the extracellular milieu. As described in this paper, although these nanomachines are typically found in enteric pathogens, their transfer into nonpathogenic *E. coli* enabled the creation of a highly flexible and adaptable platform for targeted delivery of therapeutic proteins. I plan to continue developing this and other platforms to generate custom probiotics and designer microbes to facilitate treatment of inflammatory disorders and cancer. (Read Reeves' article; DOI: 10.1021/acssynbio.5b00002).

# MELISSA TAKAHASHI



Cornell University

**Current Position.** Ph.D. candidate, Cornell University, Chemical and Biomolecular Engineering, Advisor: Julius Lucks.

**Education.** M.S. at Stanford University; B.S. at University of California at Davis.

Nonscientific Interests. I enjoy cycling, golf, exploring new places and restaurants.

My research focus is on building synthetic RNA genetic networks. To do this I have been engineering RNA transcription regulators and developing an understanding of the structural design principles that underlie their function. We now have a library of RNA regulators, and we can begin to build new synthetic networks. However, the process of testing different network designs in cells can be very slow. In this work we overcome this by adapting a cell-free transcription—translation system that allows us to test designs in 3 h as opposed to 3 days. We show the utility of the cell-free system by prototyping a new RNA networks are fast. Moving forward I'll be using this system to develop new RNA networks. (Read Takahashi's article; DOI: 10.1021/sb400206c).

# HAN XIAO



Han Xiao

**Current Position.** Associate Professor, State Key Laboratory of Microbial Metabolism, Joint International Research Laboratory of Metabolic and Developmental Sciences, and Laboratory of Molecular Biochemical Engineering, School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, China.

Education. Postdoctoral associate at the Department of Chemical and Biomolecular Engineering, University of Illinois at Urbana-Champaign. Advisor: Huimin Zhao. Ph.D. in Microbiology at the Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, China. Advisor: Sheng Yang. B.S. in Biological Science at the College of Life and Basic Sciences, Sichuan Agricultural University, China. Advisor: Tingzhao Rong and Suzhi Zhang.

Nonscientific Interests. Running, swimming, reading.

My research is focused on engineering of microbial cell factories including *Saccharomyces cerevisiae* and higher fungus for efficient production of value-added compounds such as ganoderic acids. To obtain a desired trait, multiple gene disruption is usually required. However, multiple gene disruption in *S. cerevisiae* is still labor and time intensive. In this paper, we developed a new strategy for one-step multigene disruption in *S. cerevisiae*. The efficiency of simultaneous disruption of three genes (*CAN1, ADE2* and *LYP1*) could reach as high as 87%, which enabled the identification of desired mutants by random genotyping. This strategy in *S. cerevisiae* should be a powerful tool for synthetic biology application. (Read Xiao's article; DOI: 10.1021/sb500255k).