North Carolina State University
Department of Chemical and Biomolecular Engineering

CHE 596-029: Synthetic Biology – Fall 2022

TTh 4:30-5:45p

Instructor: Dr. Albert Keung (ajkeung@ncsu.edu).

Office Hours:

Moodle Forum for Questions: There is a forum for asking questions on Moodle. The instructor will answer questions within 24 hours if possible, and students notified if the instructor will not be able to.

Guest Lectures:

Course purpose: This course is an introduction to the field of Synthetic Biology. The purpose is to provide a broad overview of the field for those interested in understanding the historical foundation, modern tools, applications, and related fields that Synthetic Biology has a potential for impact on. Other major objectives are on developing skills to critical read primary literature, and to formulate creative and rigorous research projects.

Specific Course Objectives are:

1) Refresh students on the basic principles of molecular and cellular biology.
2) Understand how quantitative approaches have been used to describe molecular enzyme kinetics, proof-reading and error correction in biological polymer, robustness of biological systems, and cell population dynamics in the contexts of antibiotic resistant bacteria and cancer.
3) Understand the systems-biology principles underlying noise, oscillations, toggle-switches, pattern formation, and other emergent properties. Become familiar with the pioneering work engineering these properties synthetically into biology.
4) Develop working knowledge of modern high throughput and efficient DNA assembly and cellular engineering methods.
5) Develop an understanding of the broad application areas of synthetic biology including cell-based cancer immunotherapy, gene drives, and metabolic engineering.
6) Understand the career opportunities in the synthetic biology arena.
7) Develop critical reading skills to rigorously analyze primary literature.
8) Develop proposal writing and project-planning skills. Have an actual proposal that could be submitted to the NIH to support student's future PhD work.

Prerequisites: A basic understanding of what cells and DNA are and a familiarity with basic single variable calculus is recommended.

Class Format: The first week will cover the basics of biology and simple math. There will be 10-20 minutes of straight lecture reviewing fundamental knowledge/background that will help you read the assigned primary literature. But please interrupt, ask clarifying questions, tell me if you're lost. Then, we will walk through the figures of the assigned papers together, discuss positive and negative aspects of the paper. We will rotate through students describing the figures. This is not meant to be scary. There is some aspect of this which is to motivate you to follow through on reading the material, but it is a safe space to learn and not understanding a figure is ok, just try your best and we'll work through them together. Strong honest effort and participation is what I am looking forward, not right answers.

Homeworks: Weekly homeworks will be due on Mondays before class through Moodle. Homeworks will cover the reading material assigned for the upcoming week and are best completed as you read the literature. Students have the opportunity to recover up to 75% of their lost points by submitting written explanations 1 week after the due date, explaining what the correct answers were after having the chance to further understand the papers after they are covered in class.

Exams: No exams. An NIH-F31 style research proposal will take the place of the final examination.

Grading:

- 30% Weekly homework questions answered on Moodle (will cover the lecture topic and the primary literature papers assigned for that week).
- 10% 1 page outline for F31 research proposal.
- 20% 6-page NIH-F31 style research proposal on a synthetic biology topic of choice.
- 20% Critiques of two of your classmates' NIH-F31 proposals.
- 20% Class participation and discussion of figures. Full credit given if you show up to class and show an honest effort to read each paper carefully. Incorrect interpretation of figures is ok and will not negatively impact your grade.
    - EoL students, please send me 2-3 questions, critiques, thoughts on the papers each week before Monday morning, and these will count as your participation grade. This way I will be able to address your questions during the class itself.
- There is no final or midterm exam. It is possible for every student in the class to earn an A. Plus/minus grading will be used.

Last Week of Class (Dead week): No test will be scheduled for the last week of class.

Academic integrity

- ***Student Code of Conduct:*** Students should refer to the University policy on academic integrity found in the Code of Student Conduct http://policies.ncsu.edu/policy/pol-11-35-01 It is the instructor's expectation that the student's name on any assignment means that they neither gave nor received unauthorized aid. Authorized aid on an assignment includes discussing the interpretation of the problem statement, sharing ideas or approaches for solving the problem, and explaining concepts involved in the problem. A good rule of thumb of what violates academic integrity is: comparison of numbers, term by term comparison and setup of equations, plagiarism. If you have trouble solving the homework independently, it is expected you seek help from the TA and the instructor or discuss the problems with classmates without unauthorized aid.
- ***Computer Work:*** Any computer work submitted must be completed on your own personal computer or from your own account. No sharing of files in any way is allowed.
- ***Course Materials****:* Sharing of any course material, including electronic or hard copies of lecture notes, homework assignments and solutions, lecture videos or recordings with third parties (i.e. anyone outside this class section), posting any materials on the internet, or allowing others to post materials on the internet or in any other public area is strictly forbidden. You may not download, post, email or otherwise share materials with a personal, public, or commercial website, or any other media.
- ***Solutions:*** While preparing homework, the use of a solution manual or equivalent (i.e. solutions that may be posted from previous class offerings or from other students at other universities) is also strictly forbidden.

Student Disabilities

- The instructor in this course will follow all applicable regulations regarding making the course accessible to students with disabilities: http://policies.ncsu.edu/regulation/reg-02-20-01 The instructor is supportive of students requiring any special accommodations. The instructor also respectfully treats any personal situation that affects the class or student learning with strict privacy.

Fellow Students in Distress

- As members of the NC State Wolfpack community, we each share a personal responsibility to express concern for one another and to ensure that this classroom and the campus as a whole remains a safe environment for learning. Occasionally, you may come across a fellow classmate whose personal behavior concerns or worries you. When this is the case, you are encouraged to report this behavior to the NC State Students of Concern website: https://ncstatecares.dasa.ncsu.edu/ 919-515-2944. Although you can report anonymously, it is preferred that you share your contact information so they can follow-up with you personally.

Course Continuity Plan

- If you are ill for a prolonged period of time, (>1 week), with doctor's note missed assignments will be excused and your grade for that component will be renormalized to exclude the missed assignments. Due dates for longer-term assignments will be pushed back to accommodate illness.
- In the cases that synchronous virtual delivery is also not possible, students will be assigned to send Dr. Keung 2

clarification questions from each previous lecture, which he will then cover in the following lecture, to promote interactivity.

Schedule

Unit 0: "Bringing everyone up to speed"

- Week 1: Crash courses on biology and math.
  - DNA, Cells, & Gene Expression
  - Algebra and single variable differential equations
  - Understanding and writing kinetic questions
  - Overview of "Synthetic Biology" topics to help you find a proposal topic.
  - Setup of Week 2: Quantifying phenotypic fluctuations in biology.

Unit 1: Historical and Quantitative Foundations of Synthetic Biology (1940-2000)

- Week 2: Quantitative analysis of fluctuations and mutations in bacteria and cancer
  - Luria SE, Delbruck M. 1943. Mutations of bacteria from virus sensitivity to virus resistance. *Genetics* 28: 491-511.
  - Fidler IJ, Kripke ML. 1977. Metastasis results from preexisting variant cells within a malignant tumor. *Science* 197: 893-5.
  - Setup of Week 3: How do cells switch phenotypes without genetic changes?
  - **HW1 Due on Moodle, 8am on Monday 8/17.**
  - **Post on the Moodle forum a list of 2 topics you are interested in writing your proposal on by 8am 8/17. I will identify a paper or two that you can work off of.**
- Week 3: Gene regulation and non-mutational phenotypic switching
  - Ferrell et al. 2014. Ultrasensitivity part I: Michaelian responses and zero-order ultrasensitivity. *Trends in Biochemical Sciences.* 39, 10:496.
  - Novick A, Wiener M. 1957. Enzyme induction as an all-or-none phenomenon. *PNAS* 43: 553-66.
  - Ptashne M. 2011. Principles of a switch. *Nature Chemical Biology* 7: 484-487.
  - Setup of Week 4: How does biology resist errors and remain robust to noise and variability?
  - **HW2 Due on Moodle, 8am on Monday 8/24.**
  - **Read the paper(s) I sent you relevant to your proposal. And schedule a 30 minute 1-on-1 Zoom meeting before 9/4 with Dr. Keung where we can discuss proposal ideas and formulate a plan.**
- Week 4: How robustness and error-correction are built into biology
  - Barkai N, Leibler S. 1997. Robustness in simple biochemical networks. *Nature* 387: 913-7.
  - Alon U et al. 1999. Robustness in bacterial chemotaxis. *Nature* 397: 168-71.
  - Hopfield JJ. 1974. Kinetic proofreading: a new mechanism for reducing errors in biosynthetic processes requiring high specificity *PNAS* 71: 4135-9.
  - Setup of Week 5: Building synthetic biological systems to understand complex biological properties.
  - **HW3 Due on Moodle, 8am on Monday 8/31.**

Unit 2: Defining Works of Modern Synthetic Biology (2000-present)

- Week 5: Engineering synthetic switches and oscillators in cells
  - Gardner TS, Cantor CR, Collins JJ. 2000. Construction of a genetic toggle switching in Escherichia coli. *Nature* 403: 339-342.
  - Elowitz MB, Leibler, S. 2000. A synthetic oscillatory network of transcriptional regulators. *Nature* 403: 335-338.
  - Setup of Week 6: We discussed engineering temporal circuits. How about spatial circuits?
  - **HW4 Due on Moodle, 8am on Monday 9/7.**
- Week 6: Engineering patterns in biological systems
  - Houchmandzadeh B, Wieschaus E, Leibler S. 2002. Establishment of developmental precision and proportions in the early *Drosophila* embryo. *Nature* 415: 798-802.
  - Basu S et al. 2005 A synthetic multicellular system for programmed pattern formation. *Nature* 434:1130-1134.
  - Setup of Week 7: We discussed engineering control over biology. How about engineering noisiness?
  - **HW5 Due on Moodle, 8am on Monday 9/14.**
  - **1 Page proposal outline due 8am Monday 9/14.**

- Week 7: Noise and stochasticity in biology
    - Elowitz MB, Levine AJ, Siggia ED, Swain PS. 2002. Stochastic gene expression in a single cell. *Science* 297: 1183-1186.
    - Eldar A, Elowitz MB. 2010. Functional roles for noise in genetic circuits. *Nature* 467: 167-173.
    - Setup of Week 8: Introduce rapid coverage of other Synthetic Biology "toy circuits".
    - **HW6 Due on Moodle, 8am on Monday 9/21.**
- Week 8: Rapidly expanding demonstrations of Synthetic Biology capabilities
    - Lecture summary of other 'toy circuits'
        - Synthetic quorum sensing: engineering cell-cell communication
        - Analog circuits, RNA devices, Circuits that count
        - Light-sensing circuits (bacterial photography)
        - Logic gates (recombinases, genetic cascades) Fussenegger
    - Sheth RU et al. 2017. Multiplex recording of cellular events over time on CRISPR biological tape. *Science.*
    - Bashor CJ et al. 2008. Using engineered scaffold interactions to reshape MAP kinase pathway signaling dynamics
    - Setup of Week 9: New molecular tools are enabling next generation synthetic devices and cells.
    - **HW7 Due on Moodle, 8am on Monday 9/28.**

Unit 3: Modern Methods to Engineer Cells and Organisms

- Week 9: DNA assembly and editing methods
    - Casini et al. 2015. Bricks and blueprints: methods and standards for DNA assembly. *Nature Reviews MCB*. 16:568.
    - Gibson DG et al. 2010. Creation of a bacterial cell controlled by a chemically synthesized genome. *Science.*
    - Bioethics of germline engineering
        - Lanphier E et al. 2015. Don't edit the human germ line. *Nature.* 519:410.
        - Ma H et al. 2017. Correction of a pathogenic gene mutation in human embryos. *Nature.* 548:413.
        - Harmon A. 2017. Human gene editing receives science panel's support. *NYTimes.*
        - NAS Germline Editing Summary. 2017.
    - Lecture summary of other topics
        - Automated assembly software and robots
        - MAGE
        - (Epi)genome engineering technologies (CRISPR, TALEs, Zinc fingers, Meganucleases)
    - Setup Week 10: Can we push past 'editing' and create new forms of life?
    - **HW8 Due on Moodle, 8am on Monday 10/5.**
    - **Six-Page Proposal Draft: Pdf Due on Moodle 8am 10/5.**
- Week 10: Biological mimics
    - Bryce E. 2017. Human 2.0: these geneticists want to create an artificial genome by synthesizing our DNA. *WIRED*.
    - Boeke J et al. 2016. The genome project-write. *Science*.
    - Roodbeen R et al. 2009. Synthetic cells and organelles: compartmentalization strategies. *BioEssays.* 31:1299-1308.
    - Choi H-J et al. 2005 Artificial organelle: ATP Synthesis from cellular mimetic polymersomes. *Nano Letters.* 5.12:2538-2542.
    - Lecture summary of other topics
        - Artificial amino acids, artificial nucleic acids
        - Cell free systems
        - Synthetic tissues/Organoids
        - Bioethics of synthetic life. Reanimation.
    - Setup of Unit 4: How can we exploit SynBio for useful purposes?
    - **HW9 Due on Moodle, 8am on Monday 10/12.**

Unit 4: Applications and the Cutting Edge

- Week 11: Gene Drives.
    - Background

        - 1x NYtimes Article. 2x Scientific American articles.
        - Burt A. 2003. Site-specific selfish genes as tools for the control and genetic engineering of natural populations. *Proc Biol Sci*. 270(1518): 921-928.
        - Esvelt KM et al. 2014. Concerning RNA-guided gene drives for the alteration of wild populations. *eLIFE*. 3:e03401.
        - Control of mosquito vectors and crop pests, microbiome engineering.
        - Real life implementations/field tests of sterile populations (Australia, Florida?)
    - Implementation
        - Hammond et al. 2016. A CRISPR-Cas9 gene drive system targeting female reproduction in the malaria mosquito vector *Anopheles gambiae*. *Nature Biotechnology*.
    - Ethical considerations. Biosecurity.
        - Oye KA. 2014. Regulating gene drives. *Science*.
    - **HW10 Due on Moodle, 8am on Monday 10/19.**
    - **Critiques of two proposals. Zoom-meet for 30 minutes to discuss critique. Due on Moodle 8am 10/19.**
- Week 12: Cell-based Cancer Immunotherapies
    - Fesnak AD et al. 2016. Engineered T cells: the promise and challenges of cancer immunotherapy. *Nature Reviews Cancer.*
    - Brentjens et al. 2013. CD19-Targeted T Cells rapidly induce molecular remissions in adults with chemotherapy-refractory acute lymphoblastic leukemia. *Science.* 5:177.
    - **HW11 Due on Moodle, 8am on Monday 10/26.**
- Week 13: Engineering cells to produce high value compounds. Metabolic Engineering.
    - Ro D-K et al. 2006. Production of the antimalarial drug precursor artemisinic acid in engineered yeast. *Nature.* 440:940.
    - Bennett M et al. 2008. Metabolic gene regulation in a dynamically changing environment. *Nature.*
    - **HW12 Due on Moodle, 8am on Monday 11/2.**
- Week 14: DNA-based Information Storage, Engineering Phage Therapies.
    - Ando et al. 2015. Engineering Modular Viral Scaffolds for Targeted Bacterial Population Editing, *Cell Systems.*
    - Koch et al. 2019. A DNA-of-things storage architecture to create materials with embedded memory, *Nature Biotechnology.*
    - **HW13 Due on Moodle, 8am on Monday 11/9.**
    - **Six-Page Final Proposal: Pdf Due on Moodle 8am on Thursday 11/12.**
- Week 15: Overview of Synthetic Biology Careers
    - Private and public biotech, agriculture, and pharmaceutical companies
    - Startups
    - Academic positions
    - Consulting
    - Venture capital and investment