

2020 syllabus. Sample draft only

USC Viterbi

BME 430 / Q BIO 430: Principles and Applications of Systems Biology

Units: 4

Lecture: Tuesdays and Thursdays, 2:00 – 3:20 pm

Discussion: Wednesdays, 4:00 – 5:50pm

Location: ONLINE only

Instructor: Professor Stacey D. Finley

Office Location: Zoom / MCB 356

Office Hours: TBD

Contact Info: Email: sfinley@usc.edu; Phone: (213) 740-8788

Emails will be replied to within 24 hours.

Teaching Assistant: Min Song

Office: Zoom / MCB 307

Office Hours: TBD

Contact Info: mins@usc.edu

Course Description

Systems biology attempts to identify interactions between the components of biological systems and understand how these interactions give rise to the physiological function of the system. This course will focus on the computational tools designed to investigate and analyze various molecular systems, including signaling pathways, metabolic systems, and gene regulatory networks.

Learning Objectives, Specific Outcomes of Instruction, and Relationship to Program Outcomes

1. Learning objectives

- Understand the principles and concepts of computational systems biology tools and analysis
- Construct and analyze mathematical models of biological systems
- Summarize and critique papers from the literature describing systems biology approaches and analyses

2. Course outcomes

- Outcome 1: Apply principles and concepts of computational systems biology tools and analysis.
- Outcome 2: Construct and analyze mathematical models of biological systems.
- Outcome 3: Summarize and apply computational theories and techniques used in systems biology.
- Outcome 4: Critically read and summarize journal articles selected from published papers in systems biology and prepare structured write-ups and oral presentations of the articles.

- Outcome 5: As part of a team, design a computational project to extend the research of a published work. Present experimental results in writing and orally.

3. Relationship between course outcomes and student outcomes

The BME Student Outcomes (a-k):

- (a) an ability to identify, formulate, and solve complex engineering problems by applying principles of engineering, science, and mathematics
- (b) an ability to apply engineering design to produce solutions that meet specified needs with consideration of public health, safety, and welfare, as well as global, cultural, social, environmental, and economic factors
- (c) an ability to communicate effectively with a range of audiences
- (d) an ability to recognize ethical and professional responsibilities in engineering situations and make informed judgments, which must consider the impact of engineering solutions in global, economic, environmental, and societal contexts
- (e) an ability to function effectively on a team whose members together provide leadership, create a collaborative and inclusive environment, establish goals, plan tasks, and meet objectives
- (f) an ability to develop and conduct appropriate experimentation, analyze and interpret data, and use engineering judgment to draw conclusions
- (g) an ability to acquire and apply new knowledge as needed, using appropriate learning strategies.

| Course outcomes↓ | Student Outcomes → | a | b | c | d | e | f | g |
|---------------------|--------------------|---|---|---|---|---|---|---|
| Outcome 1: | | X | | | X | | | X |
| Outcome 2: | | X | X | | | | | X |
| Outcome 3: | | X | | | | | X | X |
| Outcome 4: | | | | X | | | | X |
| Outcome 5: | | | | X | | X | X | X |
| All course outcomes | | X | X | X | X | X | X | X |

Course Preparation

Prerequisite: BME 210 or CHE 205; MATH 245

Recommended preparation: BISC 220

Course Notes

This course is designed to introduce you to different aspects of systems biology. Through homework assignments, exams, critical reading of primary literature, class presentations and a collaborative project, students will learn how systems biology approaches can be applied to address important biological questions. The timeline on which the material will be covered is provided below and is subject to change, at the instructor's discretion.

Web page: A class website will be setup on Blackboard containing information about the course: syllabus, reading handouts, homework assignments, grades, information about class activities, solutions to the homework sets, and an email directory of all students in the class. Use it as much as you find it useful. The web page can be accessed at: <https://Blackboard.usc.edu>.

Office Hours: Professor Finley will hold office hours every week. This is for your benefit and you should feel welcome to attend office hours as much as you need assistance. Time and location for office hours are at the beginning of the syllabus.

Technological Proficiency and Hardware/Software Required

Several practical modeling examples will be discussed, including hands-on tutorial sessions held in the Discussion period during the first two weeks of the course. Students should bring their laptops, with Matlab installed. Matlab is available from the university:

<https://software.usc.edu/index.aspx#DISTRIBUTED>.

Required Readings and Supplementary Materials

Mathematical modeling in systems biology by Brian P. Ingalls, MIT Press, 2013 (ISBN 9780262018883). Professor Finley will provide electronic copies of supplemental readings and make them available on the course website.

Description and Assessment of Assignments

Homework. Students will be responsible for homework sets that incorporate the computational modeling techniques and derivations discussed in class. These assignments are to be completed individually. Homework should be prepared on paper sheets and written legibly. Each problem must start on a new page. Units must be indicated for all numerical results. All derivations must be included with symbols before numbers are "plugged in". Homework sheets must be stapled together. The instructor is not responsible for sheets lost due to not being stapled.

Exams. The course exams will be closed-book tests consisting of quantitative problems and short essay questions. No make ups will be given. Students who are not able to attend an examination due to medical or other emergency must notify the instructor before the exam via phone message at 213-740-8788 or email at sfinley@usc.edu.

Journal club articles. Students will critically read several journal articles, selected from a list of published papers, and individually prepare a short write-up of the articles. The articles will be selected from foundational papers in systems biology describing mathematical models that are relevant to human health and disease, while still manageable for students to understand and work with (i.e., < 20 differential equations that are described in detail and can be implemented in MATLAB to reproduce the results presented in the paper). A representative example of a paper is Krishna, *et al.*, "Minimal model of spiky oscillations in NF- κ B signaling", *PNAS*, **103**, 10840–10845 (2006), which models an important transcription factor using three nonlinear ordinary differential equations. The critique should address the items listed in the rubric (see **Attachment A below**).

The journal articles will be the basis of the group presentations. In preparation for the group presentations, students should review the articles being presented and be prepared to ask 2-3 questions or discussion points regarding some component of the paper.

Group project. Students will critically read and evaluate one of the journal club articles and individually prepare a summary and critique of the paper, and all presentations should address the items listed in the rubric (see **Attachment B below**). Then, teams involving 2-4 students will work to present their journal article in class. Prior to the presentation, teams may arrange to meet with Prof. Finley to answer any questions regarding the model, its significance, and its broader implications. Additionally, throughout the semester, we will dedicate a portion of the lecture time

to the group project – to review the rubrics and expectations, check in on how teams are progressing, and discussing any issues that may arise.

Each team will work to reproduce the results from their journal club article and propose and execute a project to extend the published work. Examples of appropriate proposals for future work include: extending the model to include new species or reactions (to make the mode more physiological); performing a sensitivity analysis of the model parameters; performing bifurcation analysis on key model parameters. The rubric for the proposal is provided on the Blackboard course site, and all proposals should address the items listed in the rubric (see **Attachment B below**). A successful proposal will clearly explain two specific aims that follow logically from a limitation or gap in the existing work presented in the assigned journal article, present the methods used to complete the aims, and describe expected results (see **Attachment C below**, which is an example of a successful proposal from Fall 2014).

The research proposal, including results from reproducing the published work (“preliminary results”), will be submitted in written form. Results from the group project will be presented orally on the Final Examination date (TBA, according to the University’s Final Examination Schedule). Each student in the team must give a portion of the presentation, and the group will be evaluated as a whole, sharing the same grade. We cap the class at 20 students; thus, with teams of 3-4 students, we will have a maximum of 5 teams. By giving each team a total of ~20 minutes for their presentation (16 minutes plus an additional 3 minutes for questions), we can easily accommodate all presentations in the 120-minute period reserved for the final exam. The rubrics for the written critique and presentations are provided on the Blackboard course site (also, see **Attachments D and E below**).

Grading Breakdown

Grades will be based on the individual assignments (homework, exams, and paper summary/critique) and the group project (journal club and final presentations and research proposal).

The weighting scheme for the final grade is below:

| | |
|--------------------------------|-------------|
| Exams | 25% |
| Homework assignments | 20% |
| Written paper summary/critique | 15% |
| Participation | 10% |
| Group research project: | |
| Journal club presentation | 10% |
| Research proposal | 10% |
| Final presentation | 10% |
| <hr/> | |
| Total: | 100% |

Assignment Rubrics

Rubrics are provided as **Attachments A through E** at the end of the syllabus.

Assignment Submission Policy

Hard copy results of the homework assignments will be due by the indicated due date. Matlab code should be submitted via Blackboard by the deadline. Code must be submitted as a zipped folder with all required dependencies directly in that folder. Name the script that produces the results as “Main.m” and the folder name as “LASTNAME_HW<X>.zip”. Make sure the code works as a stand-

alone before submitting it. Points will be deducted for code that does not run. Additional directions for code submission may be provided.

Electronic versions of the research proposal and final report should be submitted via Blackboard. Hard copies are due in class on the due date.

Grading Timeline

Graded homework assignments will be returned one week after the due date. Other assignments (presentations, research proposal) will be graded and returned within two weeks of submission.

Additional Policies

Email communication. I will work hard to reply to emails within 24 hours. However, email is not the best platform to answer questions for content in this course, as typically, questions will require a detailed explanation. Therefore, students are urged to utilize office hours and discussion section, rather than relying solely on email.

Late policy. Late reports and homework will only be accepted without penalty if special circumstances exist (i.e., medical emergencies) and permission is given before the deadline. Otherwise, points will be reduced by 10% for each day it is late, starting immediately after the deadline. There are no make-up days for presentations or exams.

Electronic devices. Electronic communication devices (i.e., cell phones and iPads) must be turned off or placed away during lectures. Students are asked to refrain from using messaging apps/programs during class.

Zoom etiquette. In an effort to mimick the in-person classroom setting, I ask that all students turn on their cameras during the synchronous Zoom class sessions. Your background should be free of any inappropriate images. Please use the “raise hand” feature when you have a question. If you feel unable to adhere to these policies, please contact me prior to the class session to discuss expectations and accommodations needed.

Synchronous session recording notice. Note that the synchronous sessions will be recorded and provided to all students asynchronously.

Sharing of course materials outside of the learning environment. USC has a policy that prohibits sharing of any synchronous and asynchronous course content outside of the learning environment, as indicated below:

SCampus Section 11.12(B)

Distribution or use of notes or recordings based on university classes or lectures without the express permission of the instructor for purposes other than individual or group study is a violation of the USC Student Conduct Code. This includes, but is not limited to, providing materials for distribution by services publishing class notes. This restriction on unauthorized use also applies to all information, which had been distributed to students or in any way had been displayed for use in relationship to the class, whether obtained in class, via email, on the Internet or via any other media. (See Section C.1 Class Notes Policy).

Course Schedule (*Indicates section(s) from textbook and/or additional documents, available via Blackboard.)

| Week | Topics | Reading* | Activity |
|-----------------------|--|-------------------------------|--|
| Aug. 18 Aug. 20 | Overview and Introduction to biochemical reactions Introduction to systems biology and mathematical models Dynamic models, Mass-action kinetics | Appendix A 2.1 | HW 1 assigned |
| Aug. 25 Aug. 27 | Modeling biochemical reaction networks Timescale separation, Enzyme kinetics Regulation of enzyme activity | 2.2, 3.1 – 3.2 | HW 1 due, HW 2 assigned |
| Sept. 1 Sept. 3 | Modeling biochemical reaction networks Cooperativity, Alternative reaction mechanisms Phase planes, Direction fields, Nullclines | 3.2 | Form groups, Assign papers, Discuss group project |
| Sept. 8 Sept. 10 | Analysis of dynamic mathematical models Stability analysis Stability analysis, Bifurcation analysis | 3.3 – 3.4, Appendix C.2 | HW 2 due, HW 3 assigned |
| Sept. 15 Sept. 17 | Analysis of dynamic mathematical models Bifurcation analysis EXAM 1 | 4.1 – 4.2 | EXAM |
| Sept. 22 Sept. 24 | Analysis of dynamic mathematical models Sensitivity analysis, Parameter fitting Parameter fitting | 4.4 | HW 3 due |
| Sept. 29 Oct. 1 | Metabolic networks Graph theory Graph theory, Stoichiometric network analysis | 4.5 – 4.6 | Paper critique due, Discuss group project |
| Oct. 6 Oct. 8 | Metabolic networks Stoichiometric network analysis Modeling metabolic networks | 5.4 | |
| Oct. 13 Oct. 15 | JOURNAL CLUB PRESENTATIONS JOURNAL CLUB PRESENTATIONS | | Presentations, HW 4 assigned |
| Oct. 20 Oct. 22 | Signaling networks Network motifs of signaling systems Network motifs of signaling systems | 5.1 – 5.3 | |
| Oct. 27 Oct. 29 | Signaling systems Signal amplification, Ultrasensitivity Simple regulation | 6.1 – 6.4 | HW 4 due, HW 5 assigned |
| Nov. 3 Nov. 5 | Signaling and Gene regulatory systems Autoregulation Autoregulation | Alon handout, 7.1 – 7.4 | HW 5 due |
| Nov. 10 Nov. 12 | Signaling and Gene regulatory systems Feed-forward loops EXAM 2 | | EXAM |
| November 17, 2-4pm | FINAL PRESENTATIONS | | Research proposal due (Nov. 15), Presentations |

Discussions

The discussion section will serve to achieve three primary goals: (1) provide the opportunity to emphasize MATLAB programming concepts and their application to the systems biology principles and problems presented in the lectures; (2) provide additional time to complete more in-depth problems for which there is limited time to cover in class; and (3) enable discussion of the journal articles presented in class in greater depth.

The instructor will attend 50 minutes of each discussion on average in the semester, with the TA covering the remaining hour when the students do MATLAB exercises.

| Week | Lecture topics | Discussion topics |
|----------|---|--|
| Aug. 19 | Introduction to biochemical reactions | Introduction to MATLAB Plotting data in MATLAB |
| Aug. 26 | Modeling biochemical reaction networks | Simulating systems biology models in MATLAB |
| Sept. 2 | Regulation of enzyme activity | Evaluating published literature Presenting journal articles |
| Sept. 9 | Analysis of dynamic mathematical models | Performing phase plane analysis in MATLAB |
| Sept. 16 | Exam review | Review |
| Sept. 23 | Analysis of dynamic mathematical models: Bifurcation, Sensitivity analysis, and Parameter fitting | Performing bifurcation analysis in MATLAB Global sensitivity analysis in MATLAB Parameter estimation in MATLAB |
| Sept. 30 | Metabolic networks: Graph theory | Visualizing and analyzing graphs using Cytoscape |
| Oct. 7 | Metabolic networks: Dynamic models | Constraint-based models of metabolism |
| Oct. 14 | Journal club presentations | Student-led discussion of journal articles Discussion of research projects |
| Oct. 21 | Signaling networks: Motifs | Models of the MAPK pathway |
| Oct. 28 | Signaling systems: Analysis | Monte Carlo simulations in MATLAB |
| Nov. 4 | Gene regulatory systems: Motifs | 3-node feedforward loops |
| Nov. 11 | Exam review | Review |

Statement on Academic Conduct and Support Systems

Academic Conduct:

Plagiarism – presenting someone else’s ideas as your own, either verbatim or recast in your own words – is a serious academic offense with serious consequences. Please familiarize yourself with the discussion of plagiarism in *SCampus* in Part B, Section 11, “Behavior Violating University Standards” policy.usc.edu/scampus-part-b. Other forms of academic dishonesty are equally unacceptable. See additional information in *SCampus* and university policies on scientific misconduct, <http://policy.usc.edu/scientific-misconduct>.

Support Systems:

Student Counseling Services (SCS) – (213) 740-7711 – 24/7 on call

Free and confidential mental health treatment for students, including short-term psychotherapy, group counseling, stress fitness workshops, and crisis intervention. engemannshc.usc.edu/counseling

National Suicide Prevention Lifeline – 1 (800) 273-8255

Provides free and confidential emotional support to people in suicidal crisis or emotional distress 24 hours a day, 7 days a week. www.suicidepreventionlifeline.org

Relationship and Sexual Violence Prevention Services (RSVP) – (213) 740-4900 – 24/7 on call

Free and confidential therapy services, workshops, and training for situations related to gender-based harm. engemannshc.usc.edu/rsvp

Sexual Assault Resource Center

For more information about how to get help or help a survivor, rights, reporting options, and additional resources, visit the website: sarc.usc.edu

Office of Equity and Diversity (OED)/Title IX Compliance – (213) 740-5086

Works with faculty, staff, visitors, applicants, and students around issues of protected class. equity.usc.edu

Bias Assessment Response and Support

Incidents of bias, hate crimes and microaggressions need to be reported allowing for appropriate investigation and response. studentaffairs.usc.edu/bias-assessment-response-support

The Office of Disability Services and Programs

Provides certification for students with disabilities and helps arrange relevant accommodations. dsp.usc.edu

Student Support and Advocacy – (213) 821-4710

Assists students and families in resolving complex issues adversely affecting their success as a student EX: personal, financial, and academic. studentaffairs.usc.edu/ssa

Diversity at USC

Information on events, programs and training, the Diversity Task Force (including representatives for each school), chronology, participation, and various resources for students. diversity.usc.edu

USC Emergency Information

Provides safety and other updates, including ways in which instruction will be continued if an officially declared emergency makes travel to campus infeasible. emergency.usc.edu

USC Department of Public Safety – UPC: (213) 740-4321 – HSC: (323) 442-1000 – 24-hour emergency or to report a crime.

Provides overall safety to USC community. dps.usc.edu

ATTACHMENT A

**BME 430: Principles and Applications of Systems Biology
Rubric for Journal Club Summary / Critique**

Name: _____ Date: _____

Paper evaluated: _____

| Points possible | Item | Points assigned |
|------------------------|--|------------------------|
| 10 | Biology <i>Provide background information about the system</i> <i>Describe the importance and broader impact of the system</i> | |
| 10 | Questions / problems addressed <i>Clearly describe the questions being studied</i> <i>Explain the rationale and justification for studying the question(s)</i> | |
| 10 | Methods <i>Clearly describe the methods</i> <i>Identify the problems and limitations of the selected methods</i> <i>Explain the assumptions made</i> | |
| 30 | Results and discussion <i>Clearly describe and explain results</i> <i>Describe importance and biological significance of the results</i> <i>State the limitations of the study</i> | |
| 20 | Issues identified <i>State the limitations of the study identified by the authors</i> <i>State the limitations of the study identified by you</i> | |
| 10 | Path forward <i>Propose ideas about refining study and / or new applications of the model</i> | |
| 10 | Writing style <i>Clear, well-organized, easy to follow</i> <i>Proper grammar</i> | |
| | Total Points | ___/100 |

Additional comments and feedback:

ATTACHMENT B

**BME 430: Principles and Applications of Systems Biology
Rubric for Journal Club Presentations**

Name: _____ Date: _____

Paper evaluated: _____

| Points possible | Item | Points assigned |
|------------------------|--|------------------------|
| 10 | Biology <i>Provide background information about the system</i> <i>Describe the importance and broader impact of the system</i> | |
| 10 | Questions / problems addressed <i>Clearly describe the questions being studied</i> <i>Explain the rationale and justification for studying the question(s)</i> | |
| 10 | Methods <i>Clearly describe the methods</i> <i>Identify the problems and limitations of the selected methods</i> <i>Explain the assumptions made</i> | |
| 30 | Results and discussion <i>Clearly describe and explain results</i> <i>Describe importance and biological significance of the results</i> <i>State the limitations of the study</i> | |
| 20 | Issues identified <i>State the limitations of the study identified by the authors</i> <i>State the limitations of the study identified by the group</i> | |
| 10 | Path forward <i>Propose ideas about refining study and / or new applications of the model</i> | |
| 10 | Presentation style and Questions <i>Clear, concise slides with proper grammar and good flow.</i> <i>Presentation should be organized and appear to have been rehearsed in advance.</i> <i>Points will be deducted if presenters rely too heavily on note cards.</i> <i>The group should be able to adequately answer questions.</i> | |

Total Points ___ /100

Additional comments and feedback:

ATTACHMENT C

BME 430: Principles and Applications of Systems Biology Rubric for Research Proposals

Each team will write a research proposal that includes specific biological questions for future work that can be accomplished with mathematical analyses. The proposed work should use concepts described in class. Examples of appropriate proposals include: extending the model to include new species or reactions; performing a sensitivity analysis of the model parameters; performing bifurcation analysis on key model parameters.

Formatting requirements

Page limit: 3 pages, single-spaced includes figure(s), references limited to one additional page

Margins: No less than 0.75" around page

Font size: Arial 11pt or Times New Roman 12pt

| <u>Pts possible</u> | <u>Item</u> | <u>Pts assigned</u> |
|---------------------|---|---------------------|
| 20 | Introduction <i>Describe the biological system being studied and its importance. Cite pertinent sources from different research groups that have studied this system, and describe their results. Background information and previous work should logically lead to the specific aims Provide smooth transition into the specific aims.</i> | |
| 10 | Specific aims <i>Clearly state two well-defined, original specific aims. The aims should be built on the introduction.</i> | |
| 20 | Research methods <i>Clearly describe methods and tools needed to accomplish the aims. Present the required equations and/or theory.</i> | |
| 10 | Expected outcomes and potential results <i>Describe the reasonable results expected. Describe what you expect to learn from completing the aims. Tie the expected results to previous work from introduction section.</i> | |
| 20 | Preliminary results <i>Reproduce at least one data figure from the assigned paper. Properly label the axes and curves. Provide figure legend that describes the results. Matlab code used to generate the results will be submitted separately.</i> | |
| 10 | Conclusions <i>Summarize the proposed work. Highlight the novelty and importance of the proposed work.</i> | |
| 10 | Grammar and style <i>Clear, concise writing with good flow. Complete sentences and proper grammar. Proper headings for the sections</i> | |
| | Total Points | ___/100 |

BME 499 Research Proposal (Fall 2014)

Introduction

Amoxicillin is a commonly prescribed broad spectrum antibiotic is used to treat a variety of ailments including pneumonia, skin infections, urinary tract infections, Salmonella, Lyme disease, sickle cell anemia, and bacterial endocarditis. Amoxicillin is a 4-membered β -lactam ring, which enables it to prevent the synthesis of bacterial walls - making it a highly efficient treatment for a wide variety of bacterial infections [1]. Currently, pneumonia is the leading cause of death in children under 5, particularly in Africa and Southeast Asia. Of the 97 million pneumonia cases recorded annually, roughly 80% of the children receive improper or no treatment [2]. Our hope is that, in determining a more efficient method of producing amoxicillin, we can increase its accessibility for children with pneumonia and other bacterial infections.

In addition to amoxicillin's wide application, other advantages of this synthetic compound include high solubility and absorption properties, and stability in acidic conditions. However, the current industrial chemical process has disadvantages such as high energy costs, toxic solvents, and high waste production [3]. Instead, a milder enzyme-catalyzed pathway, which produces amoxicillin and methanol from p-hydroxyphenylglycine methyl ester (POH-PGME) and 6-aminopenicillanic acid (6-APA) using the enzyme penicillin G acylase (PGA) from *Escherichia coli* bacteria, was developed [3]. As shown in Figure 1, PGA catalyzes the main synthesis reaction (Vs) as well as two side hydrolysis reactions of the substrate and product (Vh1 and Vh2 respectively). To achieve its catalytic activity, PGA works in conjunction with ester side chains that function as acylating agents [4]. Therefore, varying the acylating agent has the potential to influence the enzymatic activity of PGA.

Goncalves et al has done extensive work in determining a model that accurately represents the reaction network for the semi-batch fed production of amoxicillin. For the purposes of their work - to determine optimal operational conditions for a single set of inputs and initial conditions - they used a hybrid neural-network model and found their input equations and parameters capable of accurately fitting experimental data. As a result, we feel confident in basing our model of amoxicillin synthesis off of their findings [3],[5].

The inspiration for our proposal comes from the work of Fernández-Lafuente et al, who observed a dual enhancing/inhibitory interaction between the methanol byproduct of amoxicillin synthesis (Figure 1) and PGA, depending on the acylating agent used [4]. Methanol has a contradictory effect when POH-PGME is the acylating agent, by increasing the synthesis/hydrolysis activity ratio of PGA with amoxicillin (vs: vh2) but decreasing the synthesis/hydrolysis activity with the POH-PGME (vs: vh1). However, methanol increases both activity ratios when mandelic acid is the acylating agent. According to Fernández-Lafuente et al., these effects can be modulated based on the PGA enzyme derivative used [4].

Specific aims

Because of the global importance of amoxicillin, our team believes further research on the dynamics of its enzymatic synthesis worthwhile. Our goal is to establish a better understanding of the reaction network and potentially elucidate conditions to maximize production of the antibiotic. To achieve this goal, our team has set forth two primary aims.

The first aim is to generate a dynamic model for the semi-batch, enzymatic synthesis of amoxicillin. To accomplish this, we will use the collection of ordinary differential equations and associated parameters generated by Goncalves et al, who previously established the quality of fit for the set of differential equations describing amoxicillin synthesis to experimental data. Using Matlab, we will simultaneously solve the differential equations for the metabolite concentrations and the sensitivity values by applying numerical differentiation methods and variable-step, variable order Taylor series methods - similar to Shirashi et al [6]. This computation also yields the dynamic logarithmic gains for the enzyme catalyzed pathway [6].

The second aim of this proposal is to apply the methodology for finding the bottleneck enzyme, as described by Shirashi et al, for the synthesis of amoxicillin [6]. Shirashi et al created their bottleneck enzyme methodology for the fermentation of Penicillin V (Pen V), which is catalyzed by three different enzymes. While PGA is the sole catalyst for amoxicillin synthesis, it has two distinct roles - as a synthetase for amoxicillin, and hydrolase for both the amoxicillin and the acylating agent (Figure 1). Because of this dual enzymatic action across three elementary reactions, we believe the techniques outlined by Shirashi et al for determining the effects of multiple enzymes on the output of a reaction network can be applied here to determine the effects of varying derivatives of PGA on amoxicillin output.

Our goal is to find the combination of PGA enzyme derivative and conditions that is the “bottleneck” - the combination to which amoxicillin production is most sensitive - using the methods set out by Shirashi et al [6]. While they analyzed a multiple-enzyme catalyzed network, the overall goal was to observe how changes in enzyme activity affected the overall output of Pen V. We are asking a similar question here: changes in which enzyme derivative have the most profound impact on amoxicillin production? If we can establish which combination results in the greatest changes in amoxicillin production, we can offer insight into how the enzymatic synthesis of amoxicillin can be optimized.

To accomplish this aim, we will vary the combination of acylating substrate (phenylglycine methyl ester or mandelic acid) and PGA derivatives (3 total) to generate parallel simulations of amoxicillin synthesis. These will be done for the non-steady state case, with infinitesimal and finite changes in enzyme activity. Using the computational methods in Shirashi et al, we will ultimately calculate the time-averaged logarithmic gains of amoxicillin production for each simulation [6]. In ranking the calculated gains of each enzyme derivative for the infinitesimal and finite changes in enzyme activity, we hope to determine the bottleneck enzyme derivative/acylating agent combination.

Research Methods

In varying the combination of acylating agent and PGA derivative, we will run a total of 6 different variations of amoxicillin synthesis. Each of these six variations will be run twice at non-steady state conditions, the first with infinitesimal changes in enzyme derivative activity and the second with finite changes. We will use equations and parameter values for amoxicillin synthesis established by Gonclaves, which apply for a semi-batch reactor at pH 6.5 and 25 C [3]. Input concentrations of PGA derivatives and acylating agents will be taken from Fernández-Lafuente et al, and methanol concentrations will be fixed at 20% in order to observe the effects of the byproduct on the catalytic activity of PGA [4].

Using the reaction model just described, we would follow the procedure outlined by Shirashi et al for determining the bottleneck enzyme [6]. First, we would calculate time-course metabolite concentrations and logarithmic gains for each simulation. Then we would compute and rank the time-averaged logarithmic gains of each simulation, for both infinitesimal and finite changes in enzyme activity. Lastly, we would assess the correlation between rankings for infinitesimal and finite changes in activity, and follow the prescribed next steps from Shirashi et al for determining the bottleneck enzyme derivative [6].

Expected outcomes and potential results

The primary outcome of the proposed study would be to ascertain if proposed methods for determining the bottleneck enzyme of a multiple-enzyme catalyzed pathway could be extended to parallel simulations of a single-enzyme catalyzed pathway. The simulation with highest ranking time-averaged logarithmic gain could be identified as the “bottleneck” enzyme derivative/acylating agent combination – meaning the combination that, when enzyme activity is changed, results in the greatest fluctuations in amoxicillin production. In order for this to occur, we expect to find some distinct variation of amoxicillin output between the different combinations of acylating agents and derivatives. This ideally would lead to a clear ranking of the time-averaged logarithmic gains for each

simulation, and therefore a clear bottleneck candidate. As seen in Shirashi et al, there may be some disagreement between infinitesimal and finite enzyme changes [6]. To rectify the inconsistency, we would have to take the further steps prescribed by Shirashi et al to see if this remedies the discrepancy [6]. Ideally, our results would establish one PGA derivative/acylating agent combination as superior to the others. However, a more reasonable outcome is eliminating less effective combinations. Further simulations and testing could be done to optimize the production of amoxicillin with this narrowed scope of useful enzyme derivatives/acylating agent combinations.

Conclusions

Our novel proposal involves two key steps: modeling the kinetic synthesis of amoxicillin and applying the methods of Shirashi et al to analyze how varying derivatives of PGA and acylating agents impact overall amoxicillin production. Rather than applying the methods for finding the bottleneck enzyme to a multi-enzyme catalyzed network, we aim to extend them to studying the impact of varying enzyme derivatives in a single enzyme catalyzed network.

The bottleneck enzyme derivative would correlate to the simulation involving the combination of acylating agent and the enzyme derivative to which the production of amoxicillin is most sensitive. By establishing this combination, it could give a target for manipulation in the large-scale enzymatic synthesis of amoxicillin that would optimize production and increase the availability of the crucial medication.

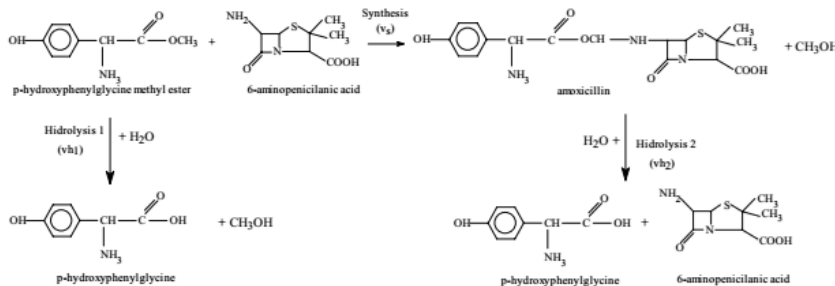
The results of the study also have the potential to inspire further research. If the validity of this extension of Shirashi et al is established, it may pertain to other single enzyme catalyzed synthesis reactions. Furthermore, establishing a functioning model of amoxicillin synthesis allows for further exploration of the system. Performing extended analyses - including sensitivity analyses and varying catalytic as well as other input parameters - will increase our understanding of the overall dynamics of amoxicillin synthesis as catalyzed by PGA.

$$\begin{aligned}
 1. \quad \frac{dCAB}{dt} &= -vAN - vAOH + FAB & 2. \quad \frac{dCAN,I}{dt} &= vAN \\
 3. \quad \frac{dCAOH,I}{dt} &= vAOH & 4. \quad \frac{dCNH}{dt} &= -vAN + FNH & 5. \quad \frac{dCAN,I}{dt} &= 0 \\
 6. \quad \frac{dCAN,S}{dt} &= vAN & 7. \quad \frac{dCAOH,L}{dt} &= 0 & 8. \quad \frac{dCAOH,S}{dt} &= vAOH \\
 9. \quad v_{h2} &= \frac{K_{cat2} * C_e * C_{an}}{K_{m2} \left(1 + \frac{C_{ab}}{K_{ab}} + \frac{C_{nh}}{K_{nh}} + \frac{C_{caoh}}{K_{caoh}} \right) + C_{an}} \\
 10. \quad v_s &= \frac{K_{m1} \left(1 + \frac{C_{an}}{K_{an}} + \frac{C_{nh}}{K_{nh}} + \frac{C_{caoh}}{K_{caoh}} \right) + C_{ab}}{K_{cat1} * C_e * C_{ab}} * \frac{C_{nh}}{K_{en} + C_{nh}} * T_{max} \\
 11. \quad v_{ab} &= \frac{K_{cat1} * C_e * C_{ab}}{K_{m1} \left(1 + \frac{C_{an}}{K_{an}} + \frac{C_{nh}}{K_{nh}} + \frac{C_{caoh}}{K_{caoh}} \right) + C_{ab}}
 \end{aligned}$$

| Parameter | Value | Parameter | Value |
|-----------|--------------------------------|-----------|-----------|
| Kcat1 | (0.18+-0.2)*10 ^A -1 | Ken | 14.4+-2.1 |
| Kcat2 | (0.33+-0.3)*10 ^A -1 | Kab | 3.78+-0.7 |
| Kas1 | 7.91+-3.6 | Kan | 9.17+-2.1 |
| Kas2 | 12.5+-3.2 | Kaoh | 10.9+-2.1 |
| Tmax | (0.61+-)*10 ^A -1 | Knh | 62+-7.2 |

Shirashi, F., and Suzuki, Y. (2009). Method for Determination of the Main Bottleneck Enzyme in a Metabolic Reaction Network by Dynamic Sensitivity Analysis. *Ind. Eng. Chem. Res.* 48: 415-423.

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Goncalves, L.R.B, Giordano R.L.C., and Giordano R.C. (2005). Mathematical modeling of batch and semibatch reactors for the enzymic synthesis of amoxicillin. *Process Biochemistry.* 40: 247-256

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- [2] Gates Foundation (2014). The Growing Market for Amoxicillin Dispersible Tablets. *US AID*: 1-21.
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- [4] Fernández-Lafuente, R., Rossel, C.M., and Guisan, J.M. (1998). The presence of methanol exerts a strong and complex modulation of the synthesis of different antibiotics by immobilized penicillin G acylase. *Enzyme and Microbial Tech.* 23: 305-310.
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ATTACHMENT E

**BME 430: Principles and Applications of Systems Biology
Rubric for Research Proposal Presentations**

Name of group members: _____

Paper assigned: _____

| Points possible | Item | Points assigned |
|------------------------|--|------------------------|
| 20 | Introduction <i>Describe the biological system being studied and its importance. Describe results from other research groups that have studied this system. Clearly explain the background information and previous work so that there is a logical progression into the specific aims.</i> | |
| 20 | Specific aims <i>Clearly state two well-defined, original specific aims. Answer the questions: what will you do and why is it important? The aims should be built on the introduction.</i> | |
| 20 | Research methods <i>Clearly describe the methods and tools needed to accomplish the aims. Present the required equations and/or theory.</i> | |
| 20 | Expected outcomes and potential results <i>Describe the reasonable results expected. Describe what you expect to learn from completing the aims. Tie the expected results to previous work described in the introduction.</i> | |
| 10 | Conclusions <i>Summarize the proposed work. Highlight the novelty and importance of the proposed work.</i> | |
| 10 | Presentation style and Questions <i>Clear, concise slides with proper grammar and good flow. Presentation should be organized and appear to have been rehearsed in advance. Points will be deducted if presenters rely too heavily on note cards. The group should be able to adequately answer questions.</i> | |

___/100

Additional comments and feedback:
