Advanced Biological Chemistry
The University of Toledo
Department of Chemistry and Biochemistry
CHEM 4500/6500/8500 and MBC 6550/8550
CRN - 44955, 46401, 44970, 44975, 41568, 41648

Instructor: Matthew Wohlever
Email: matthew.wohlever@utoledo.edu
Office Hours: MW 2:00 – 3:50 P.M. (remote)
Term: Fall 2020

Class Location: Bowman-Oddy 2059
Class Day/Time: MW 10:00 - 11:50 A.M.
Office Location: Wolfe Hall 3210B
Credit Hours: 4

COURSE/CATALOG DESCRIPTION
The chemistry of cellular and molecular transformations in biochemical systems. Molecular structure of proteins, nucleic acids, and membranes.

COURSE STATEMENT
This course will teach you how to use chemistry and biochemistry to address problems of biological significance. I will challenge you in this course to move beyond the memorization and simple application of facts to the point where you can use your knowledge to evaluate arguments in the primary literature and design experiments to advance or disprove this model.

There are things you need to memorize (such as amino acids, basics of protein structure, key experimental techniques etc.) as they are fundamental to the analysis we will be doing, but that is not the focus of the course. A good analogy is that you need to have a decent vocabulary in order to write a novel. The focus of this course is on “writing a novel”, not on learning the “vocabulary”. If you are struggling with “vocabulary”, you will need to bring yourself up to speed with this outside of class. Use your resources (office hours, text books, the library, Wikipedia, review articles, primary literature). Learning to teach yourself this “vocabulary” is an incredibly important skill that you will use throughout your life and research career. The course will be difficult not because I want to be mean, lazy, or unfair (I will provide the resources necessary for you to succeed in this course) but because this is the best way to prepare you for the tribulations of scientific research and life in general. I am training you to be my peer, and you will be treated as such. Therefore, we will be on a first name basis in the classroom.

This is an advanced course that is designed to prepare you for academic research in a biochemical lab. To receive a top grade in this course, you must move beyond learning facts and instead creatively combine what you have learned to solve research problems (i.e. design and/or trouble shoot experiments). At the end of this course, you should know how to use biochemical tools to address questions of biological significance. You should also be comfortable reading and critically evaluating the primary literature. This will require you to know the underlying assumptions with experiments and determine what conclusions can properly be made from experimental results.
This course will be divided into five sections. Section 1: Molecular structure of proteins. Section 2: Kinetics and thermodynamics of proteins. Section 3: Enzymes. Section 4: Lipids and membrane proteins. Section 5. Nucleic acids

STUDENT LEARNING OUTCOMES
A. Summarize key points in a biochemistry research article
B. Describe assumptions underlying experimental approach and evaluate if conclusions are adequately supported by data
C. Determine if experimental logic justifies the conclusions of a biochemistry research article
D. Use macromolecular structure, kinetics, thermodynamics, and molecular biology techniques to design logical experiments to test a hypothesis
E. Develop a hypothesis/model for how a biological system functions based on data from macromolecular structure, kinetics, thermodynamics, and molecular biology experiments
F. Use molecular viewing software (PyMOL) to examine a protein structure and generate a hypothesis for molecular function
H. Develop appropriate controls to show that the experiment is working as intended
I. Apply “rules of thumb” for quickly determining experimental feasibility
J. Describe expected results for experiments conducted in bulk solution compared to single molecule experiments.

PREREQUISITES AND COREQUISITES
None. However, this course will be challenging for anyone who had not passed an undergraduate biochemistry course.

REQUIRED TEXTS AND ANCILLARY MATERIALS
Technology
Due to social distancing requirements, any small group activities will be done remotely using blackboard collaborate ultra. There will be some graded quizzes on blackboard to gauge comprehension of the journal club articles.

Students will also need to download PyMOL for class. You can download the software at the links below.

Mac:
https://rocketsutoledo-my.sharepoint.com/:u:/g/personal/jbolbac2_rockets_utoledo_edu/ET8es6UJ4kdKu5JrRRBl17EBTPXEemhwvjSiiRkiqN-w?e=KGzewc

Windows:
https://rocketsutoledo-my.sharepoint.com/:u:/g/personal/jbolbac2_rockets_utoledo_edu/EXUtSBXwTkBErgRkYRLQNQ0B3wUAAzVWbUccTxTekJR0g?e=W9rODc

License File
Textbook

There is not a required text for this course. Most of the reading will be available as handouts that will be posted on Blackboard. Any general biochemistry text can be purchased if you have not had a biochemistry course previously. The syllabus will occasionally reference the Lehninger biochemistry textbook. You can get this either from the University of Toledo bookstore or buy it online from a variety of retailers. The library also has many versions of biochemistry texts available.

WEBSITE

The website for this course is available on blackboard, so you can access it with your UTAD credentials. The main page can be found at: https://blackboard.utdl.edu
You can download copies of the syllabus (if you happen to lose this one).

UNIVERSITY POLICIES

Policy Statement on Non-Discrimination on the basis of Disability (ADA)
The University is an equal opportunity educational institution. Please read The University’s Policy Statement on Nondiscrimination on the Basis of Disability Americans with Disability Act Compliance. Students can find this policy along with other university policies listed by audience on the University Policy webpage (http://www.utoledo.edu/policies/audience.html/#students).

Academic Accommodations
The University of Toledo embraces the inclusion of students with disabilities. We are committed to ensuring equal opportunity and seamless access for full participation in all courses. For students who have an accommodations memo from Student Disability Services, I invite you to correspond with me as soon as possible so that we can communicate confidentially about implementing accommodations in this course. For students who have not established affiliation with Student Disability Services and are experiencing disability access barriers or are interested in a referral to healthcare resources for a potential disability or would like information regarding eligibility for academic accommodations, please contact the Student Disability Services Office (http://www.utoledo.edu/offices/student-disability-services/) by phone: 419.530.4981 or email at StudentDisability@utoledo.edu.

Academic and Support Services
Please follow this link to view a comprehensive list of Student Academic and Support Services (http://www.utoledo.edu/studentaffairs/departments.html) available to you as a student

Harassment
The University of Toledo is committed to creating a safe environment for everyone. Every person learning, living, working and receiving health care on a University of Toledo campus has the right to a safe and secure environment, free of harassment. The University has zero tolerance for violence against members of the University community. More information can be found at Title IX office.
Safety & Health Services for UT Students
College/graduate school presents many challenges. If you are struggling with mental health, addiction, or financial issues, there are many resources on campus available to help you. Please use the following link to view a comprehensive list Campus Health and Safety Services available to you as a student.

ACADEMIC POLICIES
Academic Misconduct
I take academic misconduct very seriously. Graded problem sets are, by their very nature, open note/book. However, students must work individually on graded problem sets. You are NOT allowed to consult with others for graded problem sets FOR ANY REASON. If you need clarification on a question, please contact me. Do not put yourself or your fellow students in a compromising position by asking questions about the problem sets. All students will be required to sign an honor code for each graded assignment stating that the work is his/her own and that s/he will report any cases of academic dishonesty.

One of the most rewarding parts of college, especially graduate school, is the connections you form with your classmates. Your classmates are your future colleagues and having a broad network is vital to your success in any career, especially academic research. Therefore, you are strongly encouraged to work together on the UNGRADED problem sets. Students may also meet to discuss the papers prior to our in class journal clubs. However, students may NOT discuss the research article or the written peer review of the article that will serve as the final exam. Assignments will be checked for plagiarism using Safe Assign.

Attendance
Students are expected to attend every lecture and pay attention. Students are expected to carefully read any reading material administered during the semester. Attendance will not be formally taken, but pop quizzes and classroom participation are part of your grade.

Discussion boards
If you have a question about the course material, it’s likely that your classmates do too. Rather than e-mailing me with a question about material, please post your questions in the group discussion section on blackboard. If you e-mail me a question about the course material, I will answer it by posting in the discussion section of blackboard. For more personal matters, such as grades, students are of course welcome to contact me by e-mail. The purpose of this policy is to allow me to be a better teacher by reducing my e-mail burden. Furthermore, one of the best ways to master material is to teach it to others. You are strongly encouraged to monitor class discussions on blackboard and respond when you know the answer.

GRADING
Your final grade will be determined by the total number of points earned on graded problem sets, quizzes, literature discussions, exams, and peer review of an article in the primary literature. I will also provide ungraded problem sets for students. While these do not need to be turned in, it is strongly recommended that the students complete all of the problem sets. Below is a tentative breakdown of points for the
semester, but this is subject to change.

For your final exam you will be assigned a paper from the current literature to evaluate as if you are a peer reviewer for a journal that is considering a paper for publication. You will critically evaluate the quality of the experiments and determine if the author’s claims are fully supported by the data. This will be turned in as a short (~ 2 page) written assignment.

**Late Assignments**
Assignments are due at the beginning of class. Assignments turned in after the beginning of class are accessed a 10% penalty for being late. Assignments are then accessed an additional 10% penalty for each day that it is late. For example, if a 100 point assignment is due in class on Monday and is submitted on Tuesday, a 20 point penalty is accessed. If you need to miss class, you may submit your assignment electronically or in person BEFORE the start of class.

**Points**
- Graded problem set 1 (Sections 1 & 2) 100 points
- Graded problem set 2 (Sections 2 & 3) 100 points
- Flash Talks 100 points
- Quizzes and participation in literature discussion 100 points
- Literature Review (Comprehensive) 100 points

*Additional graded assignments may be assigned as needed. For example, pop quizzes or participation in classroom discussion may become a larger portion of the final grade if there is insufficient participation in literature days or the class shows a lack of preparation.*

**Grading**
Your final grade will be based on your percentage of the total points possible. A breakdown of the grade cut-off percentages are as follows:

- A - Total score is ≥ 85%
- B - Total score is between 75 and 84%
- C - Total score is between 65 and 74%
- D - Total score is between 55 and 64%
- F - Total score is < 55

The cut-off percentage for grades at the end of the semester may decrease, but will not increase.

**EXPECTATIONS**
There will be **NO** make-up examinations. An excused absence will be allowed only for justified and **thoroughly documented purposes**. Lack of preparation, vacation, personal problems, transportation difficulties, job conflicts, alien abduction, etc. do not constitute acceptable reasons for missing an examination.

**COURSE SCHEDULE**
Due to social distancing requirements, we are not able to do small group activities in person. Therefore, any lectures that require group work will be held as a **remote class** on blackboard collaborate ultra where we can do breakout sessions. These sessions are noted in the syllabus and will be communicated by e-mail and/or blackboard/in class announcements.

<table>
<thead>
<tr>
<th>#</th>
<th>Date</th>
<th>Topic</th>
<th>Examples &amp; Case study</th>
<th>Experimentation for Thump</th>
<th>Activities Graded &amp; Ungraded</th>
<th>References</th>
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<tbody>
<tr>
<td>Section 1 – Molecular Structure of Proteins</td>
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</table>
| 1  | 8/17  | Introduction/review of basic biochemistry  
• Syllabus, clicker  
• Instructions for downloading PyMOL  
• Introduction to the primary literature  
• Basics of protein structure  
• Amino acids  
• Basics of protein folding |  
| 2  | 8/19  | In vitro study of proteins  
• Cloning  
• Protein expression  
• Protein purification PyMOL tutorial |  
| 3  | 8/24  | Protein structure techniques  
• NMR  
• X-ray crystallography  
• Cryo-EM  
• Co-variation analysis  
• XL-MS  
• Integrative Structure Determination  
YidC |  
| 4  | 8/26  | 1st Order Reactions, Energy & Equilibrium  
• Protein folding  
• Rate laws & kinetic constants  
  o Rate constant graph from data  
  o Derive integrated sltn  
• Boltzmann distribution  
• Reaction coordinate diagrams  
• Kinetics (k) vs. Equilibrium (K) Experimental techniques for measuring 1st order reactions  
  o How to measure K_{eq}  
  o How to measure k_{f}k_{u} Limited proteolysis  
  Diffusion limit=10^{-9} W  
  fluorescence Extrapolate for K_{eq}  
  If K=1  
  Then ΔG = 1.4 kcal/mol |  
| 5  | 8/31  | Experimental techniques for measuring 1st order reactions  
• How to measure k_{f} & k_{u}  
• Half-life Experimental techniques to study protein folding  
• Point mutants, transition state, and protein folding  
• Reaction coordinate diagrams |  

References:
- Key #s in Biology Slides  
  Lehninger 1-142  
  (focus on 115-142)
- Pymol tutorial handout
- Lehninger 313-331 Rout & Sali Cell 2019
- Simple kinetics & equilibrium Handout #1  
  Lehninger 143-151
- Simple kinetics & equilibrium Handout #1
<table>
<thead>
<tr>
<th>Day</th>
<th>Date</th>
<th>Reading/Quiz</th>
<th>Journal Club</th>
<th>Ungraded Pset</th>
<th>Additional Notes</th>
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<tr>
<td>6</td>
<td>9/2</td>
<td>Journal Club #1</td>
<td>Remote Class</td>
<td>Ko et al. 2019</td>
<td>HD exchange (mass spec &amp; NMR)</td>
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<td>Methods, Supplement</td>
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<td>Split into groups, quickly summarize a figure</td>
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<td>7</td>
<td>9/9</td>
<td>Simple binding kinetics &amp; equilibria</td>
<td>Lac Operon Competing models for how repressor works</td>
<td>Multistate kinetics &amp; equilibrium handout #2</td>
<td>HD exchange</td>
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<td>How to measure $k_{on}/k_{off}$</td>
<td>Protein G &amp; Fab</td>
<td>Lehninger 158-162</td>
<td>Protein G &amp; Fab</td>
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<td>Initial rate</td>
<td>1 nM = 1 copy per E. coli</td>
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<td>Protein G &amp; Fab</td>
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<td>Pseudo dimerization</td>
<td>Data from filter binding assay</td>
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<td>Protein G &amp; Fab</td>
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<td>How to prevent rebinding</td>
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<td>$K_D$</td>
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<td>$K_D$ and fraction bound</td>
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<td>Data from filter binding assay</td>
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<td>Stoichiometry binding experiments vs. $K_D$</td>
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<td>Effective concentration</td>
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<td>Protein G &amp; Fab</td>
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<td>Review for Pset</td>
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<td>Protein G &amp; Fab</td>
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<td>8</td>
<td>9/14</td>
<td>Experimental techniques for measuring binding</td>
<td>EMSA</td>
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<td>Phizicky &amp; Fields Microbio. Rev. 1995</td>
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<td>Separation</td>
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<td>Phizicky &amp; Fields Microbio. Rev. 1995</td>
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<td>Non-separation based techniques</td>
<td>Filter binding</td>
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<td>Phizicky &amp; Fields Microbio. Rev. 1995</td>
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<td>Anisotropy</td>
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<td>Phizicky &amp; Fields Microbio. Rev. 1995</td>
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<td>9</td>
<td>9/16</td>
<td>Testing competitive vs. non-competitive inhibition with:</td>
<td>Lac Operon Competitive vs. non-competit. inhibition</td>
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<td>Lehninger 1155-1175</td>
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<td>Structure</td>
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<td>Kinetics</td>
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<td>Double mutant cycles</td>
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<td>Lehninger 1155-1175</td>
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<td>10</td>
<td>9/21</td>
<td>Literature Day - Single molecule studies of protein/DNA binding</td>
<td>Reading Quiz</td>
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<td>Donovan et. al. EMBO 2019</td>
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<td>Remote Class</td>
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<td>Donovan et. al. EMBO 2019</td>
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<td>Donovan et. al. EMBO 2019</td>
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| 11/9/23 | Complex Binding, Linked Reactions  
- Law of microscopic reversibility  
- Linked equilibrium (K1*K2 = K3*K4)  
- Cooperativity  
- Hill coefficient | Trp Repressor  
λ repressor  
Cooperativity sigmoidal, curves & semi-log plots  
Hyperbolic = 90% of binding in 100x of K_{D}  
Cooperative = 90% of binding in 10x of K_{D} |
| 11/9/28 | Allostery  
- MWC model of Allostery  
- Allosteric regulation of enzymes  
- Activation by competitive inhibitors | Hemoglobin  
pH & BPG  
Aspartate  
Transcarbam. & compet. Inhib. |
| 12/9/30 | Introduction to Enzymes  
- How enzymes catalyze reactions  
  - Stabilize transition state  
  - rxn coordinate diagrams  
  - Orient substrates  
  - Binding energy  
  - Acid-base catalysis  
Michaelis-Menten Kinetics intro assumptions | Abzymes  
Tryptsin  
Chymotrypsin  
Ungraded Pset #2 given |
| 10/5/20 | Enzyme kinetics  
- Michaelis-Menten Kinetics  
  - Derive  
- Pre-steady state kinetics  
  - Burst & lag phase kinetics  
  - Single turnover kinetics  
- Order of addition experiments  
- Necessary & sufficient  
FOF1 ATPase  
RNAP  
When K_{M} = K_{D}  
Post key for Ungraded Pset #2 after class |
| 10/7/20 | Make up lecture and review  
Remote Class | Graded Pset #2 given |
| 10/12/20 | Co-translational membrane protein insertion SEC translocon | Graded Pset #2 due |
| 10/14/20 | Post-translational membrane protein insertion  
- GET Pathway  
- EMC  
- Snd  
- SecA | Choose papers for flash talks |
| 10/14/20 | Make up lecture and review  
Remote Class | Graded Pset #2 due |

Section 3 – Enzymes

Section 4 – Lipids & Membrane Proteins

Lehninger 158-174

Lehninger 189-199
Lehninger 214-243

Lehninger 200-212


Borgese et. al. Protein J. 2019
<table>
<thead>
<tr>
<th>Week</th>
<th>Date</th>
<th>Activity</th>
<th>Notes</th>
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<tr>
<td>18</td>
<td>10/19</td>
<td>Literature Day – Membrane Protein Insertion</td>
<td>Remote Class</td>
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<td>19</td>
<td>10/21</td>
<td>Lipids and membrane protein reconstitution</td>
<td>O’Donnell et al. 2020</td>
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<td>- Lipids</td>
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<td>- Detergents</td>
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<td>Reconstitution techniques</td>
<td>Lehninger 362-369 Lehninger 377-402</td>
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<td>- ERAD</td>
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<td>- Ubiquitin proteasome system</td>
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<td>- Bag6</td>
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<tr>
<td>21</td>
<td>10/28</td>
<td>Literature Day</td>
<td>Journal Club #3</td>
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<td>Remote Class</td>
<td>Baldridge &amp; Rapoport, Cell 2016</td>
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<td>22</td>
<td>11/2</td>
<td>Flash Talks</td>
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<td>Remote Class (except for presenters)</td>
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<td>23</td>
<td>11/4</td>
<td>Flash Talks</td>
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<td>Remote Class (except for presenters)</td>
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<td>Section 5 – Nucleic acids</td>
<td>Lehninger 281-306</td>
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<tr>
<td>24</td>
<td>11/9</td>
<td>DNA/RNA structure</td>
<td>Lehninger 281-306</td>
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<td>Ribozymes</td>
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<td>Sanger sequencing</td>
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<td>Chemistry of Next Generation Sequencing</td>
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<td>11/11</td>
<td>No Class, Veterans Day</td>
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<td>25</td>
<td>11/16</td>
<td>Literature Day – Ribosome profiling</td>
<td>Journal Club #5</td>
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<td>Remote Class</td>
<td>Chen et al. 2020</td>
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<tr>
<td>26</td>
<td>11/18</td>
<td>Next generation sequencing – Guest Lecture</td>
<td>Lehninger 339-351</td>
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<td>Remote Class</td>
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<tr>
<td>27</td>
<td>11/23</td>
<td>Literature day, synthetic DNA with 8 bases</td>
<td>Journal Club #6</td>
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<td></td>
<td>Remote Class</td>
<td>Hoshika et. al. Science 2019</td>
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<td>Final</td>
<td>Peer Review of research article</td>
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<td></td>
<td>11/30</td>
<td>10:15 AM - 12:15 PM</td>
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Subject to change depending on the pace of lectures and class understanding.

**SPECIAL COURSE EXPECTATIONS DURING COVID-19**

**ATTENDANCE**
The University of Toledo has a missed class policy. It is important that students and instructors discuss attendance requirements for the course. Students must perform a daily health assessment, based on based on [CDC guidelines](https://www.cdc.gov), before coming to campus each day, which included taking their temperature. Students who are symptomatic/sick should not come to class and should contact the Main Campus Health Center at 419-530-3451. *Absences due to COVID-19 quarantine or isolation requirements are considered excused absences.* Students should notify their instructors and these absences may not require written notice.
FACE COVERINGS
All students must wear face coverings while on campus, except while eating, alone in an enclosed space, or outdoors practicing social distancing. NO students will be permitted in class without a face covering. If you have a medical reason that prevents you from wearing a face covering due to a health condition deemed high-risk for COVID-19 by the Centers for Disease Control and Prevention (CDC), you should submit a request for an accommodation through the Student Disability Services Office (SDS) by completing the online application. Students will need to provide documentation that verifies their health condition or disability and supports the need for accommodations. If a student is already affiliated with SDS and would like to request additional accommodations due to the impact of COVID-19, should contact their accessibility specialist to discuss their specific needs.

SOCIAL DISTANCING
Students should practice social distancing inside and outside the classroom please follow signage and pay attention to the seating arrangements. Do not remove stickers or tape from seats and/or tables, this is there to provide guidance on the appropriate classroom capacity based on the recommended 6 feet of social distancing between individuals. Please be conscious of your personal space and respectful of others. Also be cognizant of how you enter and exit the room; always try to maintain at least 6 feet of distance between yourself and others.