BIOCHEMISTRY 662 (BCHM 662, NUCLEIC ACIDS, MODULE II)
FALL, 2019: MWF, 9:30-10:50 P.M., BIOSCIENCE BUILDING 2118

Assoc. Prof. Jason D. Kahn, Dept. of Chemistry and Biochemistry, Univ. Maryland, College Park

Office hours: Weds. 1:30-2:30 p.m., Thurs. 2:30-3:30 p.m. (note change from 661), Chemistry 2500A

Contacting me: **jdkahn@umd.edu** much preferred to 301-405-0058. Please include "BCHM662" in your subject line and quote any previous correspondence in your emails. I will be happy to set up appointments outside of office hours if necessary.

Web and email: The course web site is available through the ELMS system (http://myelms.umd.edu). E-mail reflectors provided through Coursemail and/or ELMS will be used. It is your responsibility to make sure your e-mail address works. You should configure myelms so that you are notified by email if I post an Announcement. There is also a wealth of material at http://www.biochem.umd.edu/kahn/teach_res.

Class location: The Biomolecular Sciences Building (BSB), a.k.a. Building #296, Here it is on the official campus map, between Lot #9 and A. J. Clark Hall.

Course Description

This course is the second half of the Dept. of Chemistry and Biochemistry graduate core course on the structure and function of nucleic acids and the mechanisms of nucleic acid transactions: a biochemical approach to molecular genetics. We will cover both prokaryotic and eukaryotic systems, emphasizing common logic and mechanisms. We will emphasize recognition, bioenergetics, and fidelity. Topics are as follows:

- **RNA Biology: Transcription of DNA; regulation of gene expression by protein transcription factors and by RNA; RNA splicing, processing, and decay; RNA catalysis and the origin of life; translation of mRNA to make protein. Aspects of how these various processes interact with each other and with DNA biology, e.g. chromatin modification, DNA repair, retroviruses.**
- **Advanced methods: Selection-amplification, CRISPR/Cas, and synthetic biology.**

Assignments, Procedures, and Grading

The material for these modules is primarily lecture-based, but you may need to self-study from the literature or some of the textbooks listed below. We have not found a textbook for this class that is sufficiently useful to make you pay for it. There will be **one 1-hour exam (100 pts), four quizzes on assigned reading (25 pts each), and a final paper and presentation (150 pts)**. For exams and quizzes, you may be asked to design and interpret experiments as well as to recapitulate facts. I encourage questions and discussion in class, but class participation does not affect grading. Your course grade will be based on exam and quiz performance relative to a curve and to my expectations (*i.e.* I move borders depending on how the class as a whole did). I anticipate roughly 50:50 A:B. The curve does not require C's or worse but I will give them without hesitation if necessary. Plus/minus final grades will be given.

Academic Integrity and Other Policies

The University of Maryland, College Park has a nationally recognized Code of Academic Integrity, administered by the Student Honor Council. This Code sets standards for all undergraduate and graduate students. You are responsible for upholding these standards for this course. It is very important for you to be aware of the consequences of cheating, fabrication, facilitation, and plagiarism. For more information on the Code of Academic Integrity or the Student Honor Council, consult the Course Related Policies page at

http://www.ugst.umd.edu/courserelatedpolicies.html; it is directed to undergraduate courses but the spirit is the same, and I have not found a corresponding resource for graduate courses. To exhibit your commitment to academic integrity, you will be expected to sign the Honor Pledge on all examinations: "I pledge on my honor that I have not given or received any unauthorized assistance on this examination."

The hour exam and quizzes are "Major Graded Events." If you miss the hour exam, you must present a valid University excuse (please secure a note from the Health Services if possible). Quizzes are graded but you can self-excuse once. If you absolutely must miss the hour exam or a quiz, you must call me in advance or within 24 hours after the exam, and you must also present a valid University excuse (please secure a note from the Health Services if possible). You will then be given a makeup exam or assigned a grade based on the remaining work.

You are hereby notified that "plagiarism" will be interpreted in its broadest sense: ideas from others must be referenced; words from others must be in quotation marks and referenced. Paraphrasing without referencing will be considered plagiarism. Extensive paraphrasing from a single source is unacceptable, referenced or not. As a condition of remaining in the class, you are specifically directed to read my own web page on this: http://www.biochem.umd.edu/kahn/plagiarism.html.

Textbook: None required. References include:

Supplementary textbooks: Any recent undergraduate biochemistry text may also be useful

Bates, A. D. and Maxwell, A. (2005). *DNA Topology*. 2nd ed. Oxford University Press. Excellent monograph on this difficult topic.

Bloomfield, V.A., Crothers, D.M., and Tinoco, I., Jr. (2000). *Nucleic Acids: Structure, Properties and Functions*. University Science Books, Sausalito CA. Biophysical chemistry.

Cox, M.M., Doudna, J.A., and O'Donnell, M. (2015) *Molecular Biology: Principles and Practice*. 2nd ed. W.H. Freeman. Basically, the third half of an undergrad biochemistry text, expanded.

Craig, Nancy L., *et al*. (2014). *Molecular Biology: Principles of Genome Function*. 2nd Ed. Oxford University Press.

Atkins, J.F., Gesteland, R.F., Cech, T.R., and (2006), *RNA Worlds: From Life's Origins to Diversity in Gene Regulation*. Cold Spring Harbor Laboratory Press. Actually the 4th ed. of a classic.

Ptashne, M. and Gann, A. (2002). *Genes & Signals*. Cold Spring Harbor Laboratory Press. Heuristics of gene regulation.

Weaver, R. F. (2011). *Molecular Biology*. 5th ed. McGraw-Hill, Boston. Undergrad bio text with experiments.

Please download and install Pymol from https://pymol.org/edu/?q=educational/

Discussion Papers

We will discuss 4 papers during the module, a balance between classic papers and current research. Papers will be posted to ELMS. You are responsible for doing whatever background reading you need to in order to understand the essence of the assigned papers. We will have written quizzes in which you may be expected e.g. to answer how the experiment in Figure 3 worked (with the paper in front of you).

M. Jinek, K. Chylinski, I. Fonfara, M. Hauer, J. Doudna, E. Charpentier, "A Programmable Dual-RNA-Guided DNA Endonuclease in Adaptive Bacterial Immunity," *Science*. **337** (2012) 816–821.

X. Weng, C.H. Bohrer, K. Bettridge, A.C. Lagda, C. Cagliero, D.J. Jin, J. Xiao, "Spatial organization of RNA polymerase and its relationship with transcription in *Escherichia coli*," *Proc. Natl. Acad. Sci. USA*. **116** (2019) 20115–20123.

K. Kruger, P.J. Grabowski, A.J. Zaug, J. Sands, D.E. Gottschling, T.R. Cech, "Self-splicing RNA: Autoexcision and autocyclization of the ribosomal RNA intervening sequence of Tetrahymena," *Cell*. **31** (1982) 147–157

N. Fischer, A.L. Konevega, W. Wintermeyer, M.V. Rodnina, H. Stark, "Ribosome dynamics and tRNA movement by time-resolved electron cryomicroscopy," *Nature*. 466 (2010) 329–333.

Class Outline

1. Transcription: fundamental mechanisms — M 10/14/19
   RNA polymerases, transcription cycle, transcription bubble, supercoiling
2. Transcription in eukaryotes: fundamental mechanisms — W 10/16/19
   GTF's, multiple RNA polymerases, promoter structure
3. Transcription topics — F, 10/18/19
   (time permitting: single-molecule experiments, antitermination, cryo-EM revolution, TF/RNAP subcellular localization)
4. Regulation in prokaryotes; repression, activation, looping — M, 10/21/19
   Paradigms: lac operon, AraC, NtrC; Searching mechanisms
5. Regulation in prokaryotes continued; applications to engineering — W, 10/23/19
   Discussion of Weng et al., 2019. Quiz.
6. Interlude: The origin and development of CRISPR/Cas technology — F, 10/25/19
   History, applications, and implications of CRISPR/Cas
7. Regulation of Transcription in eukaryotes: complexity — M, 10/28/19
   Chromatin remodeling, histone code, recruitment
8. Catchup and review — W, 10/30/19
   Discussion of Jinek et al., 2012. Quiz.
9. Regulation of transcription and translation by RNA — F, 11/1/19
   RNAi, siRNA, microRNA
10. Selection-amplification methods — M, 11/4/19
    Exploring sequence space of RNA: Selex
11. RNA sensors and riboswitches — W, 11/6/19
    RNA as a small-molecule sensor and regulator

→ HOUR EXAM ← Covers through Lecture 10 — **F, 11/8/19**

12. Catalytic RNA — M, 11/11/19
    Self-splicing RNA, ribozymes, origin of life
13. RNA splicing and processing — W, 11/13/19
    Splicing mechanisms
14. Connections between RNA splicing and transcription — F, 11/15/19
15. Catchup and review — M, 11/18/19
    Discussion and quiz on Kruger,…, and Cech, 1982
16. Translation I — W, 11/20/19
    Chemistry of protein biosynthesis, ribosome structure
17. Translation II — F, 11/22/19
    The translation cycle, GTPases and ribosomal fidelity
18. Special topics in translation — M, 11/25/19
    Ribosome biogenesis, ribosomal frameshifting, ribosomopathies, antibiotic mechanisms, cotranslational folding
    Paper topics due

→ Thanksgiving ← — **W+F, 11/27+29/19**

19. Nonsense-mediated decay and other RNA degradation pathways — M, 12/2/19
    Quality control, regulation of RNA lifetime
20. Catchup and review — W, 12/4/19
    Discussion and quiz on Fischer et al., 2010
21. Systems and Synthetic Biology — F, 12/6/19
    Integration of pathways, network motifs in transcriptional regulation, engineering circuits

(**continued**)

22. Review of elaborated central dogma M, 12/9/19
    Engineering transcriptional circuits

Tuesday, December 10, 2019: Papers Due

Return and Discussion of Papers: Tuesday, 12/14/19, 8:00 a.m.–10:00 a.m.

Final Paper Assignment

Choose a primary research paper related to this class, from 2018 or 2019, from a high-profile journal like *Science*, *Cell*, *Nature*, or *PNAS*. Read the paper carefully. Track back the history of the main idea and the evolution of our understanding of it through three previous papers. For example, doing this for the Jinek paper would lead one back to the discovery of tracrRNA, then back to crRNA and the involvement of transcription in immunity, then to the discovery of CRISPR repeats as a bacterial immune system.

Timeline:

1. Email me the title and the URL of the main paper and a sentence or two describing it, on or before Monday, November 25, 2019. Please put your name in the body of the email.
2. Email me a Word document or PDF with sections 1-6 below by 11:59 p.m., Tuesday, December 10, 2019. Make sure the title of the file and the text of the file both include your name!
3. Prepare a PowerPoint describing sections 1-4 below in 4-5 slides, to be presented verbally to your classmates in 6 minutes each during the assigned final exam time 12/14/19. Email me the slides by 12/13/19 at 5 p.m. so I can assemble a big PPT rather than dealing with 16 computers.

Sections 1-4 should be a total of 4 pages including up to a total of 1 page of figures.

1. (25 pts) Summarize the central accomplishments of the main paper. This does not mean to repeat the abstract! I would like you to evaluate what made the paper worth publishing in a high-profile place – what did they really add to what was known before? If there are holes in the work, describe them.
2. (20 pts) Describe one key method that the authors used and how they evaluated the result; i.e. go into some detail in describing one figure, without just repeating the legend.
3. (30 pts) Describe the evolution of the main idea from the previous papers – what were the central insights or experiments that led to our present understanding?
4. (15 pts) Briefly discuss what the next experiments in the field are likely to be. For example, from the Jinek paper it was clear that someone was going to try to cut DNA in vivo with a chimeric RNA/Cas9.
5. (15 pts) Include a bibliography of the several papers above, formatted in the style of the bibliography of the main paper you chose, except also include the title if it is not already specified in the style.
6. (25 pts) Write a tough but fair quiz like the ones I give you, with answers.
7. (20 pts) Presentation of PowerPoint. This part of the assignment will be graded only for completion.

Learning Outcomes:

The purpose of this class is to help you learn the fundamental concepts and the analytical methods needed to understand the research literature concerning nucleic acids and molecular biology. At the end, you should be able to interpret and design experiments relevant to your own research in biochemistry and molecular biology. The purpose of classwork in graduate education is to launch you into thinking as a professional.

Syllabus Revision Date 10/14/19, version 1.00