BIOCHEMISTRY 674 (NUCLEIC ACIDS) — FALL, 2004
TuTh, 8:00-9:15 a.m., Chemistry 0127

Assoc. Prof. Jason D. Kahn, Dept. of Chemistry and Biochemistry, UMCP

Office:	Chemistry 2500A (Biochemistry, Wing 5 of the Chemistry complex)
Office hours:	Mon. 1-2 p.m., Thurs. 1-2 p.m., Chemistry 2500A; there is no TA for the course
Contacting me:	jdkahn@umd.edu much preferred to 301-405-0058. Please do not drop in to my office or lab, but I will be happy to set up appointments outside of office hours if necessary.
Web and email:	http://www.biochem.umd.edu/biochem/kahn/bchm674; there is also an e-mail reflector.

Course Description

This course concerns the structure and function of nucleic acids and the mechanisms of nucleic acid transactions: a biochemical approach to molecular genetics. We will generally cover both prokaryotic and eukaryotic systems, emphasizing common logic and mechanisms. Topics are as follows:

- **Chemistry and structure of DNA and RNA, from nucleotides to chromosomes and genomes, and some methods for studying, synthesizing, sequencing and manipulating nucleic acids. Rudimentary bioinformatics.**
- **Interactions between nucleic acids and ligands such as cations, drugs, and especially proteins.**
- **DNA Biology: Selected aspects of the biochemistry and regulation of DNA replication, transcription, recombination, and repair, and how these processes interact with each other.**
- **RNA Biology: Regulation of gene expression by RNA, RNA processing, RNA catalysis, translation.**

Procedures and Grading

This course is primarily lecture-based. Required papers from the literature will also be assigned for some lectures, as indicated on the Course Outline below. They can be read after the lecture, and study questions on the papers may be provided. All papers will be on reserve at the White Memorial Chemistry Library. I may also provide occasional handouts with extra figures and additional entries into the literature.

There will be two 75-minute exams (100 pts each), a short (7-10 pp.) paper (100 pts), and a two hour final examination (150 pts). Exams will emphasize lecture material, with some coverage of key concepts from the reading. You will be asked to design and interpret experiments as well as to recapitulate assigned material. Review sessions will be held and past exams will be available. The paper will be an examination of the historical development of our understanding of a particular topic, with emphasis on the critical experiments. I encourage questions and discussion in class, but class participation does not affect grading. The exams are quite difficult, but in the past I have had few complaints about final grades. Your course grade will be based on exam and paper performance relative to a curve and to my expectations. I anticipate roughly 50:50 A's:B's. The curve does not require C's and D's but I will give them without hesitation if necessary. Plus/minus final grades will be given.

If you absolutely must miss an hour exam, 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 assigned a grade based on the remaining exams and the paper. If you miss the final or both hour exams, you will receive a failing grade. I expect and enforce adherence to the University's Code of Academic Integrity, found at http://www.studenthonorcouncil.umd.edu/code.html. I expect you to

write out and sign the University honor pledge on each exam: "**I pledge on my honor that I have not given or received any unauthorized assistance on this assignment/examination."** Also, 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/biochem/kahn/plagiarism.html.

Please try to be on time for class. Please make sure that your cell phone does not ring during class. Cell phones must be stored out of reach and sight during exams.

Textbooks

Required: Weaver, R. F. (2002). *Molecular Biology*. 3rd ed., McGraw-Hill, Boston. Excellent source for historical and modern experiments. Also see http://www.mhhe.com/weaver3.

Bates, A. D. and Maxwell, A. (1993). *DNA Topology*. Oxford: IRL Press at Oxford University Press. 114 pp. Excellent short 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. Denoted "BTC" below

Ptashne, M. (1992). *A Genetic Switch: Phage λ and Higher Organisms*. 2nd ed. Cambridge, MA: Cell Press and Blackwell Scientific. 192 pp. Heuristics of gene regulation.

Wolffe, A. (1999). Chromatin: Structure and Function. 3rd ed. San Diego: Academic Press, Inc. 400 pp. Covers from structure to biology.

Required Papers: a balance among review articles, classic papers, and current research.

Berns, K., Hijmans, E. M., Mullenders, J., Brummelkamp, T. R., Velds, A., Heimerikx, M., Kerkhoven, R. M., Madiredjo, M., Nijkamp, W., Weigelt, B., Agami, R., Ge, W., Cavet, G., Linsley, P. S., Beijersbergen, R. L. & Bernards, R. (2004). "A large-scale RNAi screen in human cells identifies new components of the p53 pathway." *Nature* **428**, 431-437.

Bushnell, D. A., Westover, K. D., Davis, R. E., and Kornberg, R. D. (2004). "Structural basis of transcription: An RNA polymerase II-TFIIB cocrystal at 4.5 Å." *Science* **303**, 983-988.

Cosma, M. P., Tanaka, T. and Nasmyth, K. (1999). "Ordered recruitment of transcription and chromatin remodeling factors to a cell cycle- and developmentally regulated promoter." *Cell* **97**(3), 299-311.

Fischle, W., Wang, Y. & Allis, C. D. (2003). "Binary switches and modification cassettes in histone biology and beyond." *Nature* **425**, 475-479.

Moser, H. E. and Dervan, P. B. (1987). "Sequence-Specific Cleavage of Double Helical DNA by Triple Helix Formation." *Science* **238**, 645-650.

Naktinis, V., Turner, J. and O'Donnell, M. (1996). "A Molecular Switch in a Replication Machine Defined by an Internal Competition for Protein Rings." *Cell* **84**, 137-145.

Park, J.-S., Marr, M. T., and Roberts, J. W. (2002). "*E. coli* transcription repair coupling factor (Mfd protein) rescues arrested complexes by promoting forward translocation." *Cell* **109**, 757-767.

Seeman, N. C., Rosenberg, J. M., and Rich, A. (1976). "Sequence-specific recognition of double helical nucleic acids by proteins." *Proc. Natl. Acad. Sci. USA* **73**, 804-808.

Watson, J. D. and Crick, F. H. C. (1953). "Molecular Structure of Nucleic Acids: A structure for deoxyribose nucleic acid." *Nature* **171**, 737-738.

Westover, K. D., Bushnell, D. A., and Kornberg, R. D. (2004). "Structural basis of transcription: Separation of RNA from DNA by RNA polymerase II." *Science* **303**, 1014-1016.

Lecture Outline

Chapters to be read for background or amplification are specified for each lecture (W3 = Weaver, Chapter 3, etc. BCT = Bloomfield, Crothers, and Tinoco). You are not responsible on exams for material covered only in the book unless this is specifically announced. You are, however, responsible for the answers to any study questions handed out in class.

I. Nucleic Acid Sequence, Structure, and Chemistry (10 lectures)

1. Introduction and nucleic acid building blocks; W1, 2, 3 — 8/31/04
   Introduction, central dogma, nucleotide structure, primary structure, chemical stability, nomenclature
2. Structures of double helices; W2 — 9/2/04
   A, B, and Z form helices, base pairing and hydrogen bonding
   Watson and Crick, 1953
3. DNA and RNA hybridization and thermodynamics; W4,5, BCT — 9/7/04
   Base-pair stability rules, melting, hybridization, hypochromism, gene chips
4. RNA structure and triple helices; W19 — 9/9/04
   Tertiary structure and tRNA, prediction of RNA folding, antisense
   Moser and Dervan, 1987
5. Enzymatic manipulation of nucleic acids; W4, 5 — 9/14/04
   Restriction enzymes, nucleases, radiolabeling, basic genetic engineering, polymerases, PCR
6. Sequencing and synthesis of DNA and RNA; W5, 24 — 9/16/04
   DNA sequencing, chemical and enzymatic synthesis, genomics and bioinformatics
7. Chemistry: Drug and cation binding, chemical probing methods; BCT — 9/21/04
   Intercalation, groove-binding, ion atmosphere, reactivity of nucleotides, altered backbone chemistries
8. DNA bending, flexibility, and cyclization; BCT — 9/23/04
   Bending and twisting flexibility, sequence-directed bending, methods for detection and quantitation
9. Topology, supercoiling, topoisomerases; W20, BCT — 9/28/04
   Linking number, superhelix structure, topoisomerase reaction mechanisms, knots and catenanes
10. Catch-up day — 9/30/04

II. General Features of Protein-Nucleic Acid Interaction (4 lectures)

11. Biochemical methods for studying complexes; W5, 9 — 10/5/04
    Binding curves, gel mobility shift, footprinting/interference, crosslinking, filter binding, ChIP

→ EXAM I ← Covers through Section I. — **10/7/04**

12. Protein structural motifs for nucleic acid binding; W9, 12 — 10/12/04
    Helix-turn-helix, zinc fingers, bZIP proteins, TBP, hnRNP, etc.
13. Sequence- and structure-specific recognition of nucleic acids; W9 — 10/14/04
    Major groove vs. minor groove, hydrogen bonding, direct vs. indirect readout, deformability, RNA recognition
    Seeman *et al.,* 1976
14. Chromosome structure; W13 — 10/19/04
    Nucleosomes, chromatin, higher-order structure, telomeres
    Fischle et al., 2003

III. DNA Transactions (9 lectures)

15. DNA replication: fundamental mechanisms; W20, 21 — 10/21/04
    Polymerization reaction mechanisms, fidelity, structure
16. Genome replication; W20,21 — 10/26/04

Origin recognition and polymerase holoenzymes in *E. coli*; the cell cycle.
Naktinis *et al.*, 1996

17. Transcription: fundamental mechanisms; W6, W10, W11 — 10/28/04
    RNA polymerases, transcription cycle, transcription bubble, supercoiling
    Bushnell *et al.*, 2004; Westover *et al.*, 2004
18. **VOTE!!** Regulation in prokaryotes; repression, activation, looping; W7, 8 — 11/2/04
    Paradigms: lac operon, araC, ntrC. Searching mechanisms.
19. Transcription in eukaryotes: chromatin and complexity; W10, 11, 12, 13 — 11/4/04
    Holoenzyme vs. initiation complex assembly, activators, enhancers, chromatin, recruitment
    Cosma *et al.,* 1999
    I have an external commitment on Nov. 4. This lecture will be rescheduled or given by a guest.
20. Regulation of transcription by RNA; W14,W16 — 11/9/04
    RNAi, siRNA, microRNA, self-cleaving transcripts
    Berns et al., 2004 and accompanying N+V
21. Recombination; W22, 23 — 11/11/04
    Holliday junctions, recABCD, λ phage integration and excision

→ Paper Topics Due ← — **11/11/04**

→ EXAM II ← Covers through Lecture 21. — **11/16/04**

22. DNA repair; W20 — 11/18/04
    BER, NER, mismatch repair, cancer
23. "Interprocess Communication"W15.3, 20.3 — 11/23/04
    Review of regulatory and biochemical connections among replication, transcription, repair
    Park *et al.*, 2002.

→ Thanksgiving← No lecture — **11/25/04**

IV. RNA Transactions (4 lectures)

24. Catalytic RNA; W14 — 11/30/04
    Self-splicing RNA, ribozymes, origin of life
25. RNA splicing, processing, and degradation; W14, 15, 16 — 12/2/04
    Splicing mechanisms, control of mRNA lifetime, nonsense-mediated decay
26. Translation; W17, 18, 19 — 12/7/04
    Chemistry of protein biosynthesis, ribosome structure, the translation cycle

→ Paper Due ← — **12/7/04**

27. Review and/or catch up day. — 12/9/04

→ FINAL EXAM← Emphasizes 22-27 **Fri., 12/17/04, 10:30-12:30 a.m., Chem. 0127**