Overview: This is a course in the fundamentals of Molecular Biology, from a chemical and structural point of view. It is intended to provide the basic set of tools for students planning on a research career. The fundamental principles are stressed, at the expense of myriad biological details. How things are (or have been) discovered and the practice of research are emphasized. Students are responsible for knowing and understanding everything presented in lecture; the text is used as a supporting source for the material. Students are expected to attend all lectures and one discussion section per week. There will be assignments that require you to look at molecular structures using molecular graphics software that you will run on a personal computer. The required work includes problem sets, two midterms and a final exam. The class website can be found at http://bio.classes.ucsc.edu/bmb100a/

The TAs are Hossein Amiri and Eric Maklan.

Text: Cox, Doudna and O'Donnell "Molecular Biology: Principles and Practice" (Freeman) Sections of the text corresponding to each section of the course are given below.

Course Outline

I. Introduction
   Careers in BMB
   Other courses in BMB area
   B vs. MB
   Structure-Function Paradigm
   Central Dogma
   The three primary kingdoms ("prokaryotes" vs. eukaryotes)
      Biology 115 (Eukaryotic Molecular Biology)
Plan of the Course
   TAs
   Sections
   Topics
   Prerequisites
   Review
   Reading; the Text vs. Lectures
II. Nucleotides: monomeric building blocks of DNA and RNA (polymers) *(Inside front cover; Sections 3.1 and 6.1)*

the bases
- structures
- aromaticity
- tautomeric forms
- pK's
- reactivities

ribose and deoxyribose
- stereochemistry *(Section 3.4)*
- constraints of ring structure

phosphate
- phosphoester linkages and geometry
- strong negative charge

the phosphate-sugar backbone

nomenclature
- bases
- nucleosides
- nucleotides
- oligonucleotides
- polynucleotides
- torsion angles

III. Weak Interactions *(Section 3.3)*

What makes macromolecules so special? Complexity and weak interactions.

Weak relative to what?
- Covalent bond energies
- Free energy and equilibrium constants
- Bond angles
- Free rotation vs. restricted rotation

Electrostatic (Ionic, Coulombic) interactions
- Dielectric constant

Hydrogen bonding
- Donors and Acceptors
- Non-donors and non-acceptors
Bond angles
Water structure
Hydrophobic Interactions
  Van der Waals interactions
  Non-interference with water structure
  Solubilities of compounds in aqueous
    vs organic solvents
Examples
How to denature biological macromolecules

IV. How the DNA double helix was discovered *(Section 6.2; p. 210)*
  chemistry background
  Chargaff’s rules
  X-ray fiber diffraction
  densities of A and B form DNA
  model building
The DNA Story (film)
The Dickerson dodecamer *(p. 210)*
Properties of nucleic acid double helices
  The Watson-Crick base pairs
  base stacking
  A vs B
  major, minor grooves
  base tilting
  helical parameters
    screw symmetry operators
    pitch
    twist
    rise
    tilt
    roll
    propeller twist
torsion angles

V. Properties of Nucleic Acids *(Section 6.4)*
  spectra *(Fig. 6-9)*
  melting and reannealing
  hybridization

VI. DNA Replication *(Chapter 11)*
DNA is the genetic material
Avery et al.
Hershey & Chase
The Meselson-Stahl experiment
Kornberg and DNA Pol I
in vitro synthesis of DNA
Cairns' pol A mutant
DNA Pol II and DNA Pol III
Okazaki
Okazaki fragments
RNA primers
The replisome
origin
leading strand
lagging strand
DNA pol III
DNA pol I
primase
dna B
rep
ssb
ligase
DNA topoisomerases (Chapter 9)
Type I
Type II
linking numbers
twist and writhe
mechanisms
Replication in eukaryotes
Reverse transcriptase
retroviruses
RT as a tool

VII. "Recombinant" DNA: cloning, mapping and sequencing DNA (Chapter 7)
DNA cloning - useful methods
restriction endonucleases
labelling with $^{32}$P
isolation of DNA fragments by gel electrophoresis
blots - Southern, northern
ligation - sticky ends vs blunt ends
transformation
cloning vectors
plasmids
phage
cosmids
YAC vectors
how to find your clone
polymerase chain reaction (PCR)
expression
overexpression
mapping genomes
sequencing
solid-state synthesis of DNA
DNA "chips"

VIII. Mutagenesis and DNA Repair (Chapter 12)
spontaneous mutations
chemical mutagenesis
uv damage
thymine dimers
repair
excision and repair replication
photolyase
O6-methyltransferase
uvr ABC
methylation tags the reference strand

IX. Recombination (Chapters 13 and 14)
homologous recombination
recA
strand invasion
branch migration
Holliday junctions
site-specific recombination
Chi hotspots
recBC

X. Transcription (Chapter 15)
RNA polymerases
bacterial
  subunit structure
eukaryotic
  RNA pol I
  RNA pol II
  RNA pol III

initiation
  promoters
  sigma factors
  open vs. closed complexes

elongation

termination
  rho-dependent
  rho-independent
  anti-termination

XI. RNA (Section 6.3)

RNA vs DNA
  why RNA? (why DNA?)

classes of RNA
  mRNA
  tRNA
  rRNA
  snRNAs
  micro-RNAs
  SRP RNA
telomerase
  RNase P RNA
etc...

Stereochemistry of RNA
  3’ endo sugar pucker (Fig. 6-18)
  A-form helices (Fig. 6-17)

RNA can have tertiary structure

XII. The Genetic Code (Chapter 17)

The triplet code: the early days
Colinearity of the genetic map and the protein chain
  Sarabhai & Brenner
  Yanofsky
How the code was cracked
the dictionary (Inside back cover)
  sense codons
  nonsense (stop) codons
  start codons
Wobble
Exceptions to universality
Origins of the code

XIII. Translation (Chapter 18)
tRNA (Figs. 17-2, 18-13)
  modified nucleotides
  the cloverleaf secondary structure
  tertiary structure
    tertiary base-base interactions
    pi turns
    stacking
  "stretched" phosphodiester linkages
  aminoacylation
    relative free energies of amino acids,
    proteins, and aminoacyl-tRNA
    how aminoacyl tRNA synthetases
    recognize tRNA
translation mechanisms
Initiation
  Initiation factors
    IF-1
    IF-2
    IF-3
  GTP hydrolysis
  initiator tRNA
  mRNA start-site selection
  mRNA structure
  the initiation cycle
Elongation
  factors
    EF-Tu is the most abundant protein
    in the E.coli cell
    Almost all the tRNA in the cell is
    bound to EF-Tu
    EF-G
Homology to EF-Tu
Position and orientation in the ribosome
GTP hydrolysis
translocation: major molecular movement
A unifying mechanism for EF-Tu and EF-G?
hybrid states
peptidyl transferase: is it RNA?

Termination
termination codons
release factors
Direct recognition of codons by protein?

ribosomes
ribosomal RNA
primary
secondary
tertiary
ribosomal proteins
crystal and NMR structures
assembly
in vivo
in vitro
Nomura's assembly map
structure
electron microscopy
low resolution
reconstructions
difference maps
tRNAs
factors
EF-G
EF-Tu
IF-3

X-ray crystallography
difference maps of tRNAs
Translation in the context of tRNA and ribosome structure
ribosomes have tRNA binding sites
ribosomes have a mRNA binding channel (1322)
peptidyl transferase is an integral part of the ribosome
the mechanism of translation is inherent to the ribosome itself

Nonsense mutations: amber, ochre and opal
  Nonsense suppressors
    tRNA
    ribosomal RNA

Frameshift mutations
  Frameshift suppressors
    tRNA
    ribosomal RNA
  Natural frameshifting

Translational accuracy
  Drug-induced misreading
  Ram mutations
  Restrictive mutations

Antibiotics and Translation
  Streptomycin
  Chloramphenicol
  Erythromycin
  Neomycin

Translation in terms of ribosome structure

XIV. Protein Structure *(Chapter 4)*
Amino Acids: the building blocks of proteins *(Inside back cover)*
  structures
    general
      stereochemistry
      peptide bonds
    side-chains
  properties
    ionic, hydrogen bonding, hydrophobic
    S-S bridges
    pK's
    spectra

Primary Structure: amino acid sequence and other
covalent structure
  length of protein chains
  sequence determination
  DNA sequencing
  direct protein methods
separation methods
  amino acids, peptides
    amino acid analysis
  proteins
    gel electrophoresis
      1-dimensional
      2-dimensional
  columns
    sizing
    ionic
    affinity
    reverse phase (HPLC)
biological methods
  antibodies
  natural complexes
  cloning and expression

Secondary Structure: Linus Pauling’s consecutive home runs
  definition of secondary structure
  the alpha helix
    geometry
    dipole moment
    amphipathic helices
    rules for prediction
  beta pleated sheets
    parallel
    anti-parallel
    curvature: saddles and barrels
    hydrophobic and hydrophilic faces
  turns
    beta turns
      type I
      type II
      rules for prediction
    irregular turns: "loops"

Tertiary Structure
  definition
  what determines tertiary folding?
  prediction theories
    Chou-Fasman prediction
  x-ray crystallography
unit cell vs. asymmetric unit
Fourier synthesis (and analysis)
reciprocal space
amplitudes vs intensities
phase determination
modeling and refinement
domains
definition
types
  alpha
  beta-barrel
    parallel
    antiparallel
  alpha/beta
    alpha-beta-alpha sandwiches
    alpha-beta "open-face" sandwiches
  small metalloproteins
    irregular
how many kinds of domains are there?
big proteins are assembled from multiple domains
  mix-and-match
Gilbert's theory of exon shuffling

Quaternary Structure
definition
examples

XV. Transcriptional Regulation (Chapters 19, 20)
Promoters and Operators
cis-acting vs trans-acting elements
Prokaryotic transcriptional regulation
  The lac operon
  lambda repressor
    mechanism of protein-DNA recognition
    co-crystal of repressor-DNA complex
    helix-turn-helix motif
Eukaryotic transcriptional regulation
  engrailed: recognition of a eukaryotic gene
    by a helix-turn-helix motif
  enhancer elements
remote from transcriptional start
work in either orientation
require trans-acting factors

Other examples
zinc fingers
leucine zippers
TATA binding protein
recognition by beta-sheets

XVI. Introns and RNA Processing (Chapter 16)
interruption of many eukaryotic genes by introns
sequence rules for intron borders
RNA splicing
the spliceosome
snRNAs
splicesomal proteins
mechanism of splicing
lariat formation
RNA rearrangements

XVII. Eukaryotic Genomes (Chapter 10)
Chromatin
histones
nucleosome structure
nucleosome cores
higher-order structure of chromatin
Telomeres
role of telomeres
structure
telomerase
Centromeres

XIX. The RNA World: How did life begin? (Section 16.6)
The chicken-egg paradox
Alternative scenarios
So what came before RNA?
Wächtershäuser
Orgel