Study Guide

Ch. 2. noncovalent interactions: hydrogen bonds, electrostatic bonds, van der Waals interactions; be able to do calculations with pH, Henderson-Hasselbalch equation; buffers.

Ch. 3. Be able to draw the structures of the 20 amino acids at pH 7.0; know the three-letter codes and one-letter codes for the amino acids. Know which ones are acidic, basic, hydrophilic, and hydrophobic. L stereoisomers. Be able to use the Henderson-Hasselbalch equation in calculations on amino acids. Structure of the peptide bond, peptide nomenclature, N- and C-terminal; disulfide bond.

Purification of proteins: Column chromatography: ion-exchange, size-exclusion, affinity chromatography (His-tag and Ni NTA column); definition of activity, assay; calculation of yield and specific activity to monitor protein purification; SDS PAGE, log molecular weight vs. relative mobility plot can be used to determine the molecular wt. of the subunits of a protein, SDS gels can monitor purification of a protein. Know the levels of proteins structure. Primary structure = amino acid sequence. Sequencing: Edman degradation (with PITC); longer peptides are specifically cleaved with trypsin, chymotrypsin, or CNBr (know the specificities for these); protein sequence can be deduced from DNA sequence.

Ch. 4. Conformation, native conformation; protein folding is driven by burying hydrophobic residues in interior and by maximizing the number of hydrogen bonds and electrostatic interactions; peptide bond; secondary structure: α helix (what amino acids stabilize or destabilize), β pleated sheet (parallel and antiparallel); tertiary structure: prosthetic group, supersecondary structure, motifs (immunoglobulin fold), domains; protein denaturation by guanidinium, urea, β-mercaptoethanol; Anfinsen experiment, quaternary structure.

Ch. 5 recognize structure of heme (Fe^{2+} protoporphyrin IX) ; O_2 binding to myoglobin (fig. 5-5), hyperbolic O_2 binding curve (Fig. 5-4b) ; hemoglobin structure: $\alpha_2\beta_2$, sigmoidal O_2 binding curve (Fig. 5-12), isolated β chain has structure and O_2 binding like myoglobin, hemoglobin carries O_2, CO_2, and H^+; cooperativity, T state and R state, T state is stabilized by electrostatic interactions between subunits (in deoxyHb, Fe is pulled out of the plane of the heme, Fig. 5-11) BPG, O_2 binding to fetal hemoglobin; electrostatic bonds stabilize T state: BPG, CO_2, and H^+ (Bohr effect) as heterotropic allosteric effectors of hemoglobin

Antibodies, immunoglobulins, IgG; structure of IgG (Fig. 5-21), immunoglobulin fold (4-21); antigenic determinant, epitope, complementarity-determining region (CDR); polyclonal antibodies, monoclonal antibodies, procedure for making monoclonal antibodies, herceptin; Fig. 5-26a ELISA, ELISA kit for hCG, Western blot (immunoblot) (Fig. 5-26b).

Ch. 6 memorize the equations for calculating ΔG and $\Delta G'^0$ (p. 24) and be able to do the calculations; cofactor, coenzyme, prosthetic group, holoenzyme, apoenzyme; enzymes are catalysts which alter the rates of the reaction they catalyze but not the equilibrium between substrates and products or the free energy available from the reaction; transition state, activation energy; how do enzymes catalyze reactions? some enzymes form covalent bonds with the

substrate to provide an alternative pathway with a lower activation energy—using noncovalent interactions, enzymes bind the substrate in the shape of the transition state to lower the activation energy. Binding the substrate to the active site, allows specificity, entropy reduction, desolvation (excludes H_2O), and lining up of catalytic groups (induced fit); memorize the Michaelis-Menten equation, V_{max}, K_m; be able to derive V_{max} and K_m from a V vs. [S] plot or a Lineweaver-Burk plot, be able to calculate turnover number.

Reversible inhibition: competitive inhibition: inhibitor often resembles the substrate, it binds at the active site, competitive inhibition can be overcome at high substrate concentration; know what V vs. [S] plot and Lineweaver-Burk plot for competitive inhibition look like. Irreversible inhibitors: acetylcholinesterase, suicide inhibitors; transition-state analogs as enzyme inhibitors (amoxicillin/clavulanate); pH dependence of enzyme activity

allosteric: first enzyme in a pathway, usually more than one subunit, sigmoidal V vs. [S] plot, allosteric activators and inhibitors, aspartate transcarbamoylase as model (ATP, CTP); reversible covalent modification: example is phosphorylation by serine/threonine or tyrosine kinases and reversible by phosphatases; proteolytic activation, zymogens and proproteins (chymotrypsin example).

Ch 7 Monosaccharides: all sugars are D-sugars, aldose, ketose, recognize structure of glucose, fructose, ribose, and deoxyribose; hemiacetal, hemiketal, anomeric carbon, be able to recognize the structure of α and β anomers, chair form of glucose is most stable; names and abbreviations of modified monosaccharides (N-acetylglucosamine, fucose, glucuronate, N-acetylneuraminic acid or sialic acid); O-glycosidic bond, composition of sucrose, lactose, trehalose, and maltose; structure of glycogen, amylose, amylopectin, and cellulose; recognize the names of glycosaminoglycans (Fig. 7-22) and that they are components of proteoglycans (example: aggrecan), part of connective tissue and extracellular matrix; glycosyltransferases and their importance in human A, B, and O blood types; N-linked (asn) and O-linked (ser, thr) carbohydrate; Lectins: selectins are responsible for leukocyte rolling and extravasation; influenza hemagglutinin binds to sialic acid residues on cell-surface glycoproteins and neuraminidase cleaves sialic acids to release new viruses.

EXAM 2 STARTS HERE:

Ch. 8, 25 Be able to draw the structures of all the nucleosides and nucleotides and be familiar with the nomenclature (Fig. 8-4); phosphodiester linkage; Watson-Crick structure of DNA Fig. 8-13; base pairs Fig.8-11, major groove, minor groove Fig. 8-13, B-DNA and A-DNA Fig. 8-17 (dsRNA takes the A-form); DNA denaturation and annealing, Fig. 8-26. DNA replication is semiconservative, bidirectional (at 2 replication forks) from an origin of replication, 5'-3' direction, leading strand, lagging strand and Okazaki fragments; Fig. 25-5a, DNA polymerases require a template, primer with free 3' OH, 4 dNTPs; all DNA polymerases have 3'-5' exonuclease proofreading activity; processivity, DNA polymerase I has 5'-3' nuclease activity, nick translation; the β subunit of DNA polymerase III is responsible for its high processivity; replication fork: helicase, ssDNA-binding proteins, primase, DNA polymerase III (α, β, ε subunits and their functions), clamp loading complex , DNA polymerase I, DNA ligase (know

what each of these does in DNA replication); initiation is at *ori*C; elongation Fig. 25-14; termination at Ter sequences with Tus protein; cytosine deamination repair (base excision repair) Fig. 25-25; Sanger method of DNA sequencing (Figs. 8-33 and 8-34); 454 pyrosequencing (what enzymes are needed and what reactions do they catalyze?) http://www.roche-applied-science.com/publications/multimedia/genome_sequencer/flx_multimedia/wbt.htm

Ch. 26 RNA structure; mRNA, rRNA, tRNA, snRNA, miRNA and si RNA; RNA polymerases need a template and 4 NTPs but no primer, synthesis is in 5'-3'direction; template and nontemplate (coding) strands; function of σ subunit of RNA polymerase; transcription start site = promoter, the RNA polymerase binding site; components of prokaryotic promoter; a promoter sequence identical to the consensus sequence is a strong promoter; termination signals for prokaryotic transcription; eukaryotic RNA polymerase II, promoter: TATA box, Inr; TBP, TFIIs are required for the binding of RNA polymerase II to the promoter; mRNA processing in eukaryotes: 5' cap, poly A tail, splicing; know function of snRNA and snRNPs in splicing; ribozyme; complex transcripts: poly A site choice, alternative splicing. RNA-directed DNA polymerases: reverse transcriptase, telomerase.

Ch. 27 codon, initiation codon (AUG), termination codons (UAA, UAG, UGA), Wobble hypothesis; the ribosome (50S + 30S = 70S) is composed of rRNA and protein: the 16S rRNA aligns the ribosome with the Shine-Dalgarno sequence in the mRNA for initiation of translation; the 23S rRNA catalyzes the formation of the peptide bond (a ribozyme); structure of tRNAs; aminoacyl tRNA synthetases (use ATP hydrolysis to charge tRNAs with amino acid); initiating tRNAs for bacteria (formylmethionine) and eukaryotes (methionine); direction of synthesis is N to C, 5' to 3' along mRNA; Shine-Dalgarno sequence; initiation: initiation factors, GTP hydrolyzed (Fig. 27-25 what components do you need?); elongation: EF-Tu, EF-Ts, GTP hydrolyzed (27-28); peptide bond formation (27-29); translocation: EF-G, GTP hydrolyzed (27-30a); termination: release factors, RRF, EF-G (27-31); eukaryotic ribosome (60S + 40S = 80S), 28S rRNA is peptidyltransferase; eukaryotic initiation (27-27) mRNA is held in a circle by eIFs and PAB, translation starts at first AUG from 5'cap.

Ch. 9 General method of cloning (Fig. 9-1); restriction endonucleases (recognize palindromic sequences on DNA and cleave there, staggered cut generates "sticky ends"; DNA ligase; polylinker (multiple cloning site); vectors: plasmids (9-3, 9-4 how to identify bacteria containing recombinant DNA), bacteriophage λ (9-5) transform host by infection, λ replication; BAC (bacterial artificial chromosome) (9-6 and using *lacZ* gene to identify bacteria containing recombinant DNA), replication like a plasmid; for each of the vectors have a ballpark estimate of the size of foreign DNA they can accommodate, how to transform cells, and how they replicate in the host cell; expression vector (9-10) what elements are required in an expression vector? cDNA library (made from tissue mRNAs using reverse transcriptase) Why should you use a cDNA rather than a gene to make a eukaryotic protein in *E. coli*? PCR (9-16a); DNA fingerprinting using STRs; RNAi and siRNA, (p. 1145-1146 and Fig. 28-36), Dicer and RISC.

Ch. 10 Be able to draw a fatty acid given the number of carbons and double bonds and be able to give the carbon number and double bond location for a fatty acid (Table 10-1); understand the 2 ways of numbering fatty acids and what ω-3 and ω-6 signify; fatty acids contain *cis* double

bonds; length and unsaturation determine melting temperature of a fatty acid; recognize structure of membrane lipids and be able to describe their components (eg., Fig. 10-7) ; recognize glycerophospholipids PC, PE, PS, PIP_2, and cardiolipin in Fig. 10-9 ; recognize ceramide, sphingomyelin, glycolipids (cerebrosides, globosides, and gangliosides) in Fig. 10-13; cholesterol (10-17).

Ch. 11 Structure of a lipid bilayer and liposomes (Fig. 11-4); liposomes can be used for drug delivery (DaunoXome and Ambisome); nonpolar molecules can move through the bilayer rapidly, polar and charged species move slowly and require protein transporters; lateral and transverse mobility of membrane lipids (Fig. 11-16) and proteins; structure of membrane proteins (Fig. 11-7), carbohydrate is attached on the outside surface of the cell; peripheral and integral membrane proteins (Fig. 11-6); hydropathy plots (Fig. 11-11 and Table 3-1); most membrane proteins are made of α helices (Fig. 11-9); β barrel motifs are found in porins (Fig. 11-13); lipid anchors for membrane proteins, with palmitoyl, farnesyl, and GPI examples (Fig. 11-14).

uniport, symport, antiport; carriers and channels; active vs. passive transport; memorize the equation and be able to calculate ΔG for transport (eqn. 11-4); facilitated diffusion, kinetics of carrier-type transport and understand the significance of the different K_ts for glucose transport for GLUT1 and GLUT2; active transport: pumps: Ca^{2+} ATPase (SERCA) mechanism (Fig. 11-36), Na^+, K^+ ATPase (Fig. 11-37); Na^+- and H^+-driven secondary active transport (example, lactose-proton symport by lactose permease Fig. 11-43); channels catalyze passive transport of ions (Fig. 12-25); potassium channel structure (Fig. 11-48 selectivity filter); voltage-gated K^+ channels (Fig. 11-50), Na^+ channel (Fig. 12-26): mechanism of voltage gate, ion selectivity mechanism, and inactivation (Fig. 12-26); ligand-gated (structure and mechanism of acetylcholine receptor (Fig. 12-27, and class notes)).

Ch. 12. General features of signal transduction (Fig. 12-1); G protein-coupled receptors (GPCR or 7TM) with β-adrenergic receptor as example (Fig. 12-4); cAMP activates protein kinase A (Fig. 12-6); off switches: phosphatases, cyclic nucleotide phosphodiesterase, hydrolysis of GTP by $G_s\alpha$ (Fig. 12-5), Fig. 12-8; insulin receptor tyrosine kinase Fig. 12-15 through activation of Ras (monomeric G protein), Ras activates a MAPK cascade to stimulate transcription and translation, SH2 domain, and termination by phosphatases. Receptor guanylyl cyclase: YOU SHOULD KNOW NO SYNTHASE MAKES NO FROM ARGININE. NO ACTIVATES GUANYLYL CYCLASE, WHICH MAKES cGMP. cGMP activates Ca^{2+} pumps to remove calcium from the cytoplasm, cGMP IS INACTIVATED BY cGMP PHOSPHODIESTERASE (PDE). Defects in signal transduction and cancer: oncogenes, v-Src, Ras , ErbB (Fig. 12-49) EGFR and HER2 (Herceptin).

EXAM 3 STARTS HERE:

For all the pathways, know the name, location, purpose, and regulatory enzyme(s) that were given in class.

Ch. 13. ATP hydrolysis (to ADP or to AMP) drives many cell reactions; catabolism, anabolism (Fig. 3 on p. 487 and handout); recognize NAD^+/NADH, $NADP^+$/NADPH, FAD/$FADH_2$.

Ch. 14. Fig. 14-2: know names of glycolytic intermediates and be able to write out the complete pathway, where ATP is consumed and formed, where NADH is formed, and net reaction; controlling enzymes: hexokinase, phosphofructokinase-1, pyruvate kinase. Pyruvate can be converted to ethanol, lactate or CO_2 to regenerate NAD^+; oxidation to CO_2 provides much more ATP. Location of glycolysis in cell. Box 14-1: tumors use glycolysis for energy, HIF-1 stimulates synthesis of GLUT1, GLUT3, and glycolytic enzymes, ^{18}F labeled 2-fluoro-2-deoxyglucose is used to detect tumors in PET scans.

Gluconeogenesis makes glucose from the noncarbohydrate precursors lactate, glycerol, propionate, and amino acids. Located in the mitochondrion, cytosol, and endoplasmic reticulum; know which enzymes in gluconeogenesis bypass the irreversible steps of glycolysis (Fig. 14-16); pyruvate carboxylase contains biotin and is a regulatory enzyme, fructose 1,6-bisphosphatase is a regulatory enzyme; stoichiometry of gluconeogenesis (Eqn. 14-9 on p. 556).

Pentose phosphate pathway: located in cytosol, glucose 6-phosphate dehydrogenase is regulatory enzyme; oxidative branch: glucose 6-phosphate is converted to ribose 5-phosphate and 2 NADPH are produced; nonoxidative branch: 3 C_5 intermediates are converted to 2 C_6 and C_3.

Ch. 15. glycogen degradation: located in cytosol; be able to describe the structure of glycogen; degradation: glycogen phosphorylase (regulatory enzyme) (phosphorolytic cleavage, Fig. 15-25) and debranching enzyme and phosphoglucomutase. glycogen synthesis: located in cytosol; UDP-glucose is "activated" form of glucose used for synthesis; UDP-glucose pyrophosphorylase, glycogen synthase (regulatory enzyme, Fig. 15-30); branching enzyme; glycogenin (Fig. 15-33). Regulation of glycogen phosphorylase: Fig. 15-35 (GPCR regulation by reversible covalent modification), Fig. 15-34.

Ch. 16. pyruvate dehydrogenase complex: eqn in Fig. 16-2, be able to recognize thiamine pyrophosphate, lipoamide, FAD, coenzyme A; pyruvate dehydrogenase complex is a regulatory enzyme.

citric acid cycle: acetyl CoA is oxidized to 2 CO_2 with the formation of 3 NADH, 1 $FADH_2$, 1 GTP; intermediates in the citric acid cycle are used to synthesize other biological molecules; be able to name all the intermediates in the citric acid cycle in order and know at which steps NADH, $FADH_2$, GTP, and CO_2 are formed (Fig. 16-7); regulatory enzymes are citrate synthase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase.

anaplerotic reactions (example, pyruvate carboxylase reaction). Recognize structure (Fig. 16-16) and know function of biotin.

Ch. 17. mobilization of stored triacylglycerols (Fig. 17-3): glucagon and epinephrine activate triacylglycerol lipase using cAMP as a second messenger, perilipin; glycerol can be converted to DHAP for gluconeogenesis; serum albumin

fatty acids are attached to CoA in the cell by fatty acyl-CoA synthetase; carnitine acyltransferase I is the regulatory enzyme for fatty acid oxidation; fatty acids are transported into the mitochondrion as carnitine derivatives in exchange for carnitine (Fig. 17-6).

β oxidation: during each round of oxidation, 1 acetyl CoA, 1 $FADH_2$, and 1 NADH are formed; acetyl CoA is oxidized to 2 CO_2 in the citric acid cycle; odd-chain fatty acids are oxidized until propionyl CoA is left-the propionyl CoA is converted to succinyl CoA in a biotin- and coenzyme B_{12}-dependent pathway; recognize coenzyme B_{12}

ketone bodies: acetoacetate, β-hydroxybutyrate, and acetone are produced from acetyl CoA; they can be used as fuel by skeletal muscle, heart, renal cortex, and (during starvation) the brain; Fig. 17-20; ketone bodies are overproduced in untreated type 1 diabetes mellitus and during starvation due to an excess of glucagon (stimulating gluconeogenesis, which uses oxaloacetate and slows the citric acid cycle, and stimulating TAG breakdown and fatty acid oxidation, which produces acetyl CoA.)

Ch. 18. Know the alanine aminotransferase and aspartate aminotransferase reactions; pyridoxal phosphate is the prosthetic group (it is involved in many reactions of amino acid chemistry: aminotransferases, racemases, decarboxylases), be able to recognize its structure (Fig. 18-5a). Glutamate dehydrogenase (Fig. 18-7); Fig. 18-8, glutamine synthetase and glutaminase reactions; glucose alanine cycle (Fig. 18-9) glutamine and alanine are nontoxic N carriers in blood. Urea cycle: Fig. 18-10 and name, location, purpose, and regulatory enzyme. Recognize structures of biotin, tetrahydrofolate, and S-adenosylmethionine. Fig. 18-15: know the definition of glucogenic and ketogenic amino acids, know one amino acid for each intermediate. Phenylalanine hydroxylase deficiency results in phenylketonuria, it is detected in newborns and alleviated by using a low phenylalanine diet; Fig. 18-27: MITV are degraded to propionyl CoA and then succinyl CoA in the coenzyme B_{12}-dependent pathway.

Ch. 19. mitochondrion structure (Fig. 19-1); be able to identify the structures of ubiquinone, hemes of the cytochromes, and iron sulfur centers; reactions of mitochondrial electron transport as given in lecture; be able to write out electron transport from NADH to O_2 and from succinate to O_2 with the complete names of complexes and their order, coenzyme Q, and cytochrome c, proton pumping by complexes I, III, and IV; be able to calculate ΔG for proton transport; chemiosmosis: proton pumping drives ATP synthesis (Fig. 19-19); structure of ATP synthase and mechanism (Fig. 19-25 and 19-26); Fig. 19-32: hypoxia increases ROS, HIF-1 causes inhibition of pyruvate dehydrogenase to slow the formation of NADH and $FADH_2$, and causes conversion of cytochrome oxidase to a form better suited for low oxygen conditions and reduce the formation of ROS. thermogenin (UCP) (Fig. 19-34); superoxide and peroxide are reactive oxygen species (ROS) produced by the metabolism of O_2 and are removed by superoxide dismutase, glutathione peroxidise, and catalase; apoptosis and cytochrome c, Fig. 19-37.

NEW MATERIAL FOR FINAL EXAM STARTS HERE:

For all the pathways, know the name, location, purpose, and regulatory enzyme(s) that were given in class.

Ch. 19 structure of chloroplast; recognize chlorophyll; light harvesting complex. In photosynthetic electron transport, water is oxidized to O_2 and NADPH and ATP are formed; components of the photosynthetic electron transport in order including H_2O, oxygen-evolving complex, photosystem II, plastoquinone, cytochrome bf complex, plastocyanin, photosystem I, ferredoxin, ferredoxin-$NADP^+$ oxidoreductase, $NADP^+$; the oxygen-evolving complex contains manganese; Fig. 19-63; Fig. 19-64.

Ch. 20 Fig. 20-14 the Calvin cycle (also name, purpose, location in the cell, and regulatory enzyme), located in chloroplast stroma; rubisco is the regulatory enzyme and has carboxylase and oxygenase activities; phosphoglycolate from oxygenase activity must be salvaged; C_4 plants incorporate CO_2 into C_4 intermediates in the mesophyll cells, these are transported to bundle sheath cells where they generate CO_2 for the Calvin cycle there, and this minimizes the oxygenase activity of rubisco (Fig. 20-23).

Ch. 21 Fatty acid synthesis: located in the cytosol, acetyl CoA carboxylase is the regulatory enzyme and contains biotin; malonyl CoA is used in synthesis, fatty acid synthase, intermediates are carried on acyl carrier protein (ACP), 2 NADPH required per round; to make palmitate, need 8 acetyl CoA, 7 ATP, and 14 NADPH; citrate carries acetate groups (8) to cytosol, and this process generates 8 NADPH.

All 27 C atoms of cholesterol come from acetyl CoA; location: cytosol, regulatory enzyme, HMG CoA reductase; know the pathway of cholesterol synthesis by the number of carbons as shown in class, recognize the structure of isopentenyl pyrophosphate (be able to spell it right), importance of farnesylation; steroids, bile salts, and vitamin D are made from cholesterol.

Ch. 22 *Rhizobium* symbiosis (what the plant supplies, what the bacteroid supplies, leghemoglobin); stoichiometry of the nitrogenase complex (p. 854); nitrogenase complex is composed of dinitrogenase reductase (Fe protein) and dinitrogenase (MoFe protein); reactions catalyzed by glutamine synthetase (22-1) and glutamate synthase (22-2); Fig. 22-9 know one amino acid for each precursor and that pentose phosphate pathway, glycolysis and citric acid cycle intermediates are used to synthesize amino acids; know the amino acids which are used to synthesize neurotransmitters and which neurotransmitters they make, and that pyridoxal phosphate is involved (Fig. 22-29).

Purine synthesis (Fig. 22-32-know where the atoms in the purine ring come from): the base is built on the sugar (phosphoribosyl pyrophosphate, PRPP) in the cytosol; gln-PRPP amidotransferase is a regulatory enzyme. Pyrimidine synthesis: the base is formed and then added to PRPP in the cytosol; aspartate transcarbamoylase is the regulatory enzyme; know the source of the atoms in the pyrimidine ring; ribonucleotide reductase; thymidylate synthase pathway (Fig. 22-44).

Ch. 23 For insulin and glucagon, know their effect on blood glucose, their mechanism of signal transduction; insulin stimulates storage and synthesis pathways; glucagon stimulates energy

mobilization pathways; mechanism of insulin release by the pancreas in response to high blood glucose (handout and class notes, Fig. 23-28). Metabolism in Type 1 and Type 2 diabetes mellitus; sulfonylureas and metformin are used to treat type 2 diabetes, know their mechanism of action. Body mass regulation: leptin is produced by adipocytes and stimulates the hypothalamus to produce anorexigenic peptides (suppress eating) and stimulates sympathetic neuron signals (see handout and class notes for norepinephrine pathway, Fig. 23-25b) to cause TAG breakdown and the synthesis of UCP to promote thermogenesis and fatty acid oxidation.