GRADUATE OFFERING OF MACROMOLECULAR STRUCTURE AND FUNCTION, BIOSC 2810
FALL TERM, 2015; updates to the syllabus will be provided electronically on Courseweb

INSTRUCTOR	Dr. Paula Grabowski Email: pag4@pitt.edu Office hours will be held in Langley Lobby: Day/time TBD, or by appointment (see Courseeweb)
TEACHING ASSISTANTS	Graduate TA, Lynley Doonan; email: LMD82@pitt.edu UTA, Chris Chung Office hours will be held in Langley Lobby: Day/time TBD (see Courseweb)
CLASS MEETINGS	Lectures: MWF 1:00 – 1:50 PM, 169 Crawford Hall Recitations: Thurs 12:00-12:50 & 1:00-1:50 PM, 241 Crawford Hall
COURSE DESCRIPTION	Biochemistry is a logical way of thinking about, and experimentally probing, life's processes at the scale of atoms and molecules. In this course we will explore macromolecular structure, function and dynamics of protein and RNA molecules by reasoning through fundamental concepts and experimental approaches. In addition to textbook readings, you will have exposure to the scientific literature throughout the course for real world illustrations of experimental tools and approaches. Pre-requisites: BIOSC 160 & 2-semesters of Organic Chemistry
COURSE OBJECTIVES	***1. Chemical reasoning – you will understand the language and reasoning of the following core concepts*** a. Macromolecular structure/function: levels of protein structure, motifs, conformational changes, allosteric movements, folding/unfolding; Chromophore structure/function b. Receptor-ligand interactions: stereochemistry; equilibrium dissociation constants c. Enzyme mechanisms and inhibition at the active site, including substrate and transition state analogs, suicide inhibitors, feedback inhibition; RNA enzymes: structure/function/evolution; roles in biology and infectious disease d. Membrane chemistry & architecture; active and passive transport ***2. Experimental reasoning – you will understand the principles of the following tools and approaches and be able to apply your understanding to interrogate the structure and function of biological macromolecules*** a. Protein purification and analytical separation methods, including column chromatography, isoelectric focusing, and ultracentrifugation b. Protein sequencing and analysis, including MALDI and ESI mass spectrometry, Sanger's reagent; proteases; bioinformatics resources; FRET sensors c. X-ray diffraction as an approach to atomic level structure/function; use of transition state analogs to pinpoint or modulate the active site d. Michaelis-Menten kinetics as an approach to understanding enzyme mechanism, including the use of steady state approximation, kcat, Km, and kcat/Km parameters; pH dependence of enzymatic reactions e. Ribozyme engineering through SELEX
PRE-REQUISITES	Why are TWO semesters of Organic Chemistry with a grade of C or better required as pre-requisites? To equip you with a working knowledge of chemistry for a successful outcome this semester.
COURSEWEB	Course materials including lecture notes will be posted electronically on CourseWeb. You will be responsible for checking this site regularly for updates, assignments, and other postings.
THE FOLLOWING READING MATERIALS ARE REQUIRED:	• Principles of Biochemistry, by Lehninger, Nelson and Cox, 6th Edition, 2013. *Copies of the textbook are on reserve in Langley Library. It is very important to use the 6th edition!* • Research papers (POSTED PAPERS) will be assigned as REQUIRED supplementary reading throughout the course. These will be provided to you as links or postings on CourseWeb. • ***Do the reading assignments (and problems) before coming to lecture/recitation. Bring these materials with you to class!***
GRADING	Your grade in BIOSC 2810 will be determined by the sum of the following components (600 points in total): In-class quizzes 240 points (40%), Final exam 120 points (20%), Assignments 140 points (23%), and one NEWS & VIEWS-style paper on a topic to be announced, 100 points (17%). Final grades will be assigned based on the following scale: percentages in the 90's = A range; 80's = B range; 70's = C range; 60's = D range; 50 or below = F.

	QUIZZES (4 x 60 points each): • Quizzes will be closed book with no aids permitted (calculators or electronic devices). • Quizzes must be completed in pencil only. Do not use pen or marker. • Questions of clarification are not permitted during a quiz. • If you miss a quiz you will need to have a compelling excuse in the form of written documentation, such as a signed letter from a physician or the equivalent. The instructor will make the determination of whether or not a make-up quiz will be given, and the format/date/time of the make-up. If the excuse is not acceptable, a grade of zero will be recorded. **FINAL EXAM (120 points):** • The Final Exam will be given at the date and time scheduled by the university Registrar. Rescheduling is not permitted, except under the following circumstance. For students who have three Final Exams (including the one for this class) scheduled on the same day: If you would like to request permission to reschedule the final exam for this class, you must do so in writing in accordance with the policies of the Registrar. • The Final Exam is required, and will be cumulative. • The Final Exam will not be returned to you, but you will be permitted to view your exam by appointment. **ASSIGNMENTS/PROJECTS (140 points; 12 x 8 points + 44):** Written assignments will be completed outside of the classroom. Follow the instructions as given. • How will your assignments be graded? The following components will be evaluated: ○ On-time submission is required; late assignments will not be graded. ○ Completeness; If your work is incomplete/deficient, or if you exceed the page limit, points will be deducted from your score. ○ Reasoning; show or explain your reasoning clearly. ○ Originality; copying from another source or another student's work without acknowledging that source is considered plagiarism and will be classified as a major deficiency and such assignments will not be graded. ○ If your work is of exemplary quality (more than expected), you will receive one or two additional points. ***NEWS & VIEWS-STYLE PAPER (100 points):*** *You will write a News and Views-style paper on a research paper or topic chosen by the instructor: first draft of paper 25 points; peer review 20 points; response to peer review 20 points, Final draft of revised paper, 35 points.*
ACADEMIC INTEGRITY	Students in this course will be expected to comply with the University of Pittsburgh's Policy on Academic Integrity Any student suspected of violating this obligation for any reason during the semester will be required to participate in the procedural process, initiated at the instructor level, as outlined in the University Guidelines on Academic Integrity. This may include, but is not limited to, the confiscation of the examination of any individual suspected of violating University Policy. Furthermore, no student may bring any unauthorized materials to an exam, including dictionaries and programmable calculators.
DISABILITY SERVICES	If you have a disability for which you are or may be requesting an accommodation, you are encouraged to contact both your instructor and Disability Resources and Services (DRS), 140 William Pitt Union, (412) 648-7890, drsrecep@pitt.edu, (412) 228-5347 for P3 ASL users, as early as possible in the term. DRS will verify your disability and determine reasonable accommodations for this course.
COPYRIGHT NOTICE	Course materials may be protected by copyright. United States copyright law, 17 USC section 101, et seq., in addition to University policy and procedures, prohibit unauthorized duplication or retransmission of course materials. See Library of Congress Copyright Office and the University Copyright Policy.
STATEMENT ON CLASSROOM RECORDING	***To ensure the free and open discussion of ideas, students may not record classroom lectures, discussion and/or activities without the advance written permission of the instructor, and any such recording properly approved in advance can be used solely for the student's own private use.***

SCHEDULE OF TOPICS & ASSIGNMENTS

	Dates	Lecture Topics	Reading Assignments
Week 1	M 8/31	Welcome; amino acid chemistry; non-covalent interactions in proteins; amphipathic compounds; UV VIS spectroscopy	Ch3.1, BOX 3-1, Fig10-23
	W 9/2	Amino acids & peptides; amino acids capabilities as nucleophiles; aspirin & acetylation of COX; biomolecules derived from amino acids	Ch3.1; Fig 13-2,3; Fig 6-9; Fig 21-15; Fig22-29; Fig22-31
	Th 9/3	RECITATION PG: acid base chemistry of amino acids; amino acid salt bridges in the protein core; Tryptophan & ion channel proteins	Ch2 prob 15, 32, Fig 3-11; Fig 11-13
	F 9/4	Henderson-Hasselbalch; peptide bond formation/hydrolysis; Glutathione Bioinformatics of the week: ***NCBI PUBCHEM & 3D VIEWER***	Ch3.2
Week 2	M 9/7	*Labor Day Holiday*	
	W 9/9	Protein purification: cell extracts, column chromatography, HPLC, use of affinity tags for protein purification_Fig9-11	Ch3.3 & Ch3 prob 15 Ch9_Fig 9-11
	Th 9/10	RECITATION: net electric charge of peptides; isoelectric focusing	Ch3 prob 4, 5, 11, 22
	F 9/11	Insulin: structure/function (Fig 3-24), Insulin family signature sequence, Bioinformatics of the week: ***PROSITE*** (http://prosite.expasy.org); P01308; ***MORPH SERVER AT YALE BIOINFORMATICS***; Insulin morph ID 940260-22867	Ch3.4; BOX 23-1; Ch3 prob 21 & 22
Week 3	M 9/14	Proteases (Table 3-6); Mass Spectrometry; Proteomics (Snapshot MS)	Ch3 Fig 3-30, 3-31; ***SNAPSHOT***
	W 9/16	Protein secondary and tertiary structure; domain structure/function	Ch4.1-4.3; ***KLENOW Fig 2a***
	Th 9/17	RECITATION: secondary structure, CD spectra, *KLENOW Fig 2a*	Ch4 prob 1, 2, 4, 10; ***KLENOW HODGKIN***; BOX4-5 & BOX17-2
	F 9/18	Intro to X-ray crystallography; Dorothy Crowfoot Hodgkin & vitamin B12 (also see: http://www.nobelprize.org, and search for Dorothy Hodgkin)	Also see PubChem
Week 4	M 9/21	Protein motifs and conformational changes, EF-hand motifs; Calmodulin; sequence logos; http://molmovdb.mbb.yale.edu/molmovdb/	Ch4.1-4.3 & Box3-2 **MORPH SERVER at Yale**
	W 9/23	Factors contributing to the correct folding of polypeptide chains; scrambled Ribonuclease & kinetics of renaturation; denaturation curves	Ch4.4 & ***ANFINSEN***
	Th 9/24	RECITATION: secondary structure; sequence logos & PROSITE; ANFINSEN	Ch4 prob 13, 14 & ***ANFINSEN***
	F 9/25	A short story of Aequorin; protein purification from 50 tons of jellyfish! Bioinformatics of the week: ***NCBI STRUCTURE DATA BANK***	***SHIMOMURA*** & review Ch3.3
Week 5	M 9/28	**QUIZ 1 (will cover material up to and incl. Th 9/24)**	
	W 9/30	GFP structure/function: ORMO study design, results & validation; mass spectrometry; GFP chromophore; cyclization of Ser-Tyr-Gly	***ORMO Figs 1 & 2*** & BOX 12-3
	Th 10/1	RECITATION: GFP continued; Greek Key motifs; site-directed mutagenesis	***ORMO Table 2*** & BOX 12-3
	F 10/2	Fluorescence Resonance Energy Transfer (FRET); FRET Sensors	***HEIM Fig 2; TSIEN slides***
Week 6	M 10/5	Receptor-ligand interactions; equilibrium dissociation constants	Ch5.1-5.2, Table 5-1, WE5-1
	W 10/7	Immunoglobulin fold; monoclonal antibodies & binding complementarity	*Fig 5-21,*
	Th 10/8	RECITATION: Morphine Fab 9b1 (PDB ID, 1Q0Y); working with Kd's; binding complementarity	Ch5 prob 1, 4, 10, 11
	F 10/9	Antibodies of note: Herceptin; role in breast cancer therapeutics	TBA
Week 7	M10/12	**QUIZ 2 (will cover material from W 9/30 to Th 10/8)**	
	W10/14	Enzymes; rate enhancement by entropy reduction; transition states	Ch6.1,6.2
	Th10/15	RECITATION: Enzyme workshop part 1	Ch6 prob 4, 5, 7; Figs6-5 & 6-7
	F10/16	Enzyme kinetics and inhibition; steady state, kcat, Km, kcat/Km, Part 1	Ch6.3
Week 8	M 10/19	*Fall Break; class moved to Tuesday*	
	T 10/20	Enzyme kinetics and inhibition; steady state, kcat, Km, kcat/Km, Part 2	Ch6.3, ***XIE***
	W10/21	Ornithine decarboxylase mechanism & Trojan horse inhibitors	Ch6.3, BOX6-3
	Th10/22	RECITATION: Enzyme workshop part 2	Ch6 prob 8, 11, 12
	F 10/23	Chymotrypsin: pH activity profile; steady state kinetic analysis	Ch6.4
Week 9	M10/26	Chymotrypsin mechanism: role of Ser95-His57-Asp102; kinetics	Ch6.4
	W10/28	Enzyme regulation by phosphorylation/dephosphorylation; Protein Kinase A catalytic & regulatory subunits; why nature chose phosphates!	Ch6.4, Fig 12-6; ***HUNTER***
	Th10/29	RECITATION: Development of protein kinase inhibitors to combat cancer	BOX 12-5 (Imatinib)
	F 10/30	Pyrimidine biosynthesis & role of ATCase; feedback inhibition	Fig 6-33 & Fig 22-38
Week 10	M 11/2	**QUIZ 3 (will cover material from W 10/14 to F 10/29)**	
	W 11/4	Allosteric enzyme regulation: Aspartate Transcarbamoylase (ATCase); subunit composition; role of Zn2+; ultracentrifugation (Fig 6!)	***GERHARDT***, Figs 3-6

	Th11/5 F 11/6	RECITATION (ATCase freeze frame project) ATCase: site-directed mutagenesis and kinetic analysis	 ***LIPSCOMB***
Week 11	M11/9 W11/11 Th11/12 F 11/13	Catalytic RNA structure/function; alkaline hydrolysis of RNA; base-paired helical structures and pseudoknots; ***MFOLD WEBSERVER FOR FOLDING RNA MOLECULES*** (http://mfold.rna.albany.edu/?q=mfold) Crawling out of the RNA World: evolutionary perspectives on ribozymes and RNP enzymes; catalytically active pseudoknots in biology & disease RECITATION; The 50S Ribosome peptidyl transferase activity The 50S Ribosome peptidyl transferase activity, continued	Ch8.1-8.2; Figs 8-8, 8-24, 8-25, 26-28; 26-29 ***CECH Fig 1; STAPLE*** ***NISSEN*** ***FEDOR***
Week 12	M 11/16 W11/18 Th11/19 F11/20	TWISTER PROJECT PART 1: TWISTER PROJECT PART 2: RECITATION: MORE ON TWISTER! Systematic Evolution of Ligands by EXponential enrichment (SELEX); RNA aptamers	***ROTH*** ***ROTH*** ***ROTH*** ***SASSANFAR***, BOX 26-3
Week 13	M 11/23 W11/25	Lipids, prostaglandins & NSAIDs; working with lipids ***Thanksgiving recess, Wednesday 11/25 – Sunday 11/29***	Ch10.3-10.4; Ch prob 20
Week 14	M 11/30 W 12/2 Th12/3 F 12/4	Membrane architecture; membrane spanning proteins; hydropathy plots ***QUIZ 4 (will cover from W 11/4 to M 11/18)*** RECITATION: Glucose transporter Fig 11-30, 11-31, **11-32** Introduction to ion channels; how ion channels open & close; transmembrane topology; selectivity; gating	Ch11.1-11.2 Ch 11 prob 15, 17, 22 ***ASHCROFT*** Figs 1&2; Ch 11, pp 420-424
Week 15	M12/7 W 12/9 Th12/10 F12/11	K channels: Selectivity filter hypothesis; sequence alignment of K channels K channels: architecture and function of selectivity filter; study design & use of monoclonal antibody Fab fragments; mechanism RECITATION: review ion channels and channelopathies Active transport: Na+K+ ATPase Pump structure/function, Jens Skou	Ch 11.3, ***DOYLE*** *Fig 1* ***ZHOU,*** Figs 1-5 ***ASHCROFT, ZHOU*** Fig 11-36; 11-37; Ch 11 prob 8
FINAL		**FRI DEC 18 FROM 4:00-5:50 PM, 169 Crawford Hall**	

2015 READING LIST – primary research papers, authoritative reviews, and commentaries
*****All entries are required reading**

Topic	Short name	Full article citation	comments
Proteases, protein domains	KLENOW ***Fig 2a and text!***	Klenow H, Overgaard-Hansen K. Proteolytic cleavage of DNA polymerase from Escherichia Coli B into an exonuclease unit and a polymerase unit. FEBS Lett. 1970 Jan 15;6(1):25-27. PubMed PMID: 11947326.	Classic structure function paper identifies multiple functions in one polypeptide!
	HODGKIN **Figs 2, 4, 8, and Fig 12! Read all!**	Hodgkin DC. The x-ray analysis of complicated molecules. Science. 1965 Nov19;150(3699):979-88. Review. PubMed PMID: 5320817.	Amazing account of the use of x-ray crystallography
Mass spectro-metry	SNAPSHOT MS	Leitner A, Aebersold R. SnapShot: mass spectrometry for protein and proteome analyses. Cell. 2013 Jul 3;154(1):252-252.e1. doi: 10.1016/j.cell.2013.06.025. PubMed PMID: 23827686	A useful collection of info/resources about mass spec
Green Fluorescent Protein	GFP Nobel and Tsien slides	GFP: Discovery, Expression and Development, supplied as link: http://www.nobelprize.org/nobel_prizes/chemistry/laureates/2008/advanced.html; (slides from Roger Tsien Nobel lecture, FRET and FRET sensors)	Great overview of major discoveries associated with GFP
	SHIMOMURA	Shimomura O., Biol Bull. 1995 Aug;189(1):1-5. A short story of aequorin. PMID: 7654844 [PubMed - indexed for MEDLINE]	Discovery of Aequorin & GFP
	ORMO ***Figs 1& 2, Table 2***	Ormö M, Cubitt AB, Kallio K, Gross LA, Tsien RY, Remington SJ. Crystal structure of the Aequorea victoria green fluorescent protein. Science. 1996 Sep 6;273(5280):1392-5. PubMed PMID: 8703075; http://www.ncbi.nlm.nih.gov/pubmed/?term=8703075	Atomic structure of GFP and chromophore; a challenging paper!
	HEIM **Fig 3!**	Heim R, Tsien RY. Engineering green fluorescent protein for improved brightness, longer wavelengths and fluorescence	Initial evidence for use of GFP and

		resonance energy transfer. Curr Biol. 1996 Feb 1;6(2):178-82. PubMed PMID: 8673464.	derivatives as FRET sensors
Protein folding	ANFINSEN ***Figs 1-3 and text involved***	Anfinsen CB. Principles that govern the folding of protein chains. Science. 1973 Jul 20;181(4096):223-30. PubMed PMID: 4124164 ***(read the first section on scrambled ribonuclease and the disturbing observation about the kinetics of refolding ribonuclease in vivo versus in vitro!)***	Classic paper on principles of protein folding and discovery of protein disulfide isomerase
Enzyme kinetics Short review	XIE ***Read all!***	Xie XS. Biochemistry. Enzyme kinetics, past and present. Science. 2013 Dec 20;342(6165):1457-9. doi: 10.1126/science.1248859. Erratum in: Science. 2014 Apr 25;344(6182):360. PubMed PMID: 24357307.	Brief synopsis of enzyme kinetics; a helpful review of basic concepts!
Protein kinases	HUNTER **Read all of this review!**	Hunter T. Why nature chose phosphate to modify proteins. Philos Trans R Soc Lond B Biol Sci. 2012 Sep 19;367(1602):2513-6. doi: 10.1098/rstb.2012.0013. Review. PubMed PMID: 22889903; PubMed Central PMCID: PMC3415839.	Commentary on protein phosphorylation
ATCase	LIPSCOMB ***Figs 1-5; Fig 7 (PALA), Fig 10 (role of Arg54) and related text***	Lipscomb WN, Kantrowitz ER. Structure and mechanisms of Escherichia coli aspartate transcarbamoylase. Acc Chem Res. 2012 Mar 20;45(3):444-53. doi: 10.1021/ar200166p. Epub 2011 Oct 19. Review. PubMed PMID: 22011033; PubMed Central PMCID: PMC3276696.	Excellent review of ATCase; a challenging paper; role of Arg 57
	GERHARDT ***Figs 3-5, and Fig 6!***	Gerhart JC, Schachman HK. Distinct subunits for the regulation and catalytic activity of aspartate transcarbamylase. Biochemistry. 1965 Jun;4(6):1054-62. PubMed PMID: 5320387.	Classic paper on subunit structure of ATCase
RNA WORLD	CECH ***Fig 1; read all!***	Cech TR. Crawling out of the RNA world. Cell. 2009 Feb 20;136(4):599-602. doi: 10.1016/j.cell.2009.02.002. PubMed PMID: 19239881.	Perspectives on the RNA World hypothesis
RIBOZYMES	FEDOR	Fedor MJ, Williamson JR. The catalytic diversity of RNAs. Nat Rev Mol Cell Biol. 2005 May;6(5):399-412. Review. PubMed PMID: 15956979.	Review of catalytic RNAs
PSEUDO-KNOTS	STAPLE ***Figs 1-2; read first two pages***	Staple DW, Butcher SE. Pseudoknots: RNA structures with diverse functions. PLoS Biol. 2005 Jun;3(6):e213. Epub 2005 Jun 14. PubMed PMID: 15941360; PubMed Central PMCID: PMC1149493.	RNA pseudoknots in biology and infectious disease
SELEX & TWISTER	SASSANFAR ***All Figs and text***	Sassanfar M, Szostak JW. An RNA motif that binds ATP. Nature. 1993 Aug 5;364(6437):550-3. PubMed PMID: 7687750.	SELEX case study
	ROTH ***All figs & suppl figs and text***	Roth A, Weinberg Z, Chen AG, Kim PB, Ames TD, Breaker RR. A widespread self-cleaving ribozyme class is revealed by bioinformatics. Nat Chem Biol. 2014; Jan;10(1):56-60. doi: 10.1038/nchembio.1386. Epub 2013 Nov 17. PubMed PMID: 24240507; PubMed Central PMCID: PMC3867598.	TWISTER discovery & structure function
K Channels	ZHOU ***All figs and text!***	Zhou Y, Morais-Cabral JH, Kaufman A, MacKinnon R. Chemistry of ion coordination and hydration revealed by a K+ channel-Fab complex at 2.0 A resolution. Nature. 2001 Nov 1;414(6859):43-8. PubMed PMID: 11689936. (challenging!)	Classic paper by MacKinnon of the atomic structure of the selectivity filter with K ions
	DOYLE ***Fig 1 and text***	Doyle DA, Morais Cabral J, Pfuetzner RA, Kuo A, Gulbis JM, Cohen SL, Chait BT, MacKinnon R. The structure of the potassium channel: molecular basis of K+ conduction and selectivity. Science. 1998 Apr 3;280(5360):69-77. PubMed PMID: 9525859.	Protein alignments support the hypothesis of the selectivity filter
	ASHCROFT ***Figs 1-2; Tables 1-2*** ***Read all!***	Ashcroft FM. From molecule to malady. Nature. 2006 Mar 23;440(7083):440-7. Review. PubMed PMID: 16554803.	Authoritative review of ion channel structure/function