BCHM669B Methods for Protein Structure Determination FALL-2002

Course meets in CBSO 2103, MoFri 9:30-10:45 am

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This is a graduate level course designed as an introduction to modern methods for biomolecular structure determination at atomic level resolution. The course will cover the theory and basic principles underlying of the two major high-resolution experimental methods: X-ray crystallography and nuclear magnetic resonance (NMR), and some theoretical methods for protein structure prediction and protein molecular dynamics simulations. The course will start with a review of physico-chemical properties of biomacromolecules, their composition, and principles of their three-dimensional architecture. While all the above-mentioned methods will be covered, the in-depth emphasis will be on the determination of protein structure and dynamics in solution using NMR. The students will learn NMR pulse sequence design (including product operator formalism), principles of multidimensional NMR, methods for protein signal assignment and structure calculation, and "model-free" analysis of protein dynamics. In addition to theoretical studies the students will get hands-on experience in NMR data analysis and in simulation of the outcome of NMR experiments on a computer.

Course prerequisites: calculus, undergraduate level biochemistry and physical chemistry.

Topics:

1. *Proteins*:
   composition, interactions, structure, and architecture of protein fold.
2. *Crystallography*:
   X-ray diffraction, physical principles, von Laue conditions, structure factor equation, symmetries and space groups, real vs. reciprocal space, from crystal to structure, phase problem, beyond X-ray: neutron diffraction, electron microscopy.
3. *Nuclear Magnetic Resonance, basics*:
   general principles of NMR spectroscopy, classical description, Bloch's equations, chemical shift, quantum mechanical description of NMR spectroscopy, spin-Hamiltonian, eigenstates, transitions, product operator formalism and how to use it.
4. *Multidimensional NMR Spectroscopy*:
   From 1-D to 2-D to n-D, homonuclear coherence transfer and mixing: COSY, NOESY, TOCSY; heteronuclear coherence transfer: INEPT, HSQC, HMQC, TROSY.
5. *Experimental aspects of NMR*:
   quadrature detection, sign discrimination, coherence selection, phase cycling, gradients; data processing: window function, zero-filling, linear prediction, isotope filtering/editing; computer simulations of the outcome of NMR experiments using the "Virtual NMR Spectrometer" program.
6. *NMR for biomolecular structure determination*:
   NOEs, J-couplings, H-bonding; spin system assignment, NOESY signal assignment, triple-resonance methods for spin system typing, sequential assignment; 2^o structure

prediction (J-couplings, Karplus equation, H-D exchange, CSI); from NOEs to structure, example of structure assignment and calculation.

7. *NMR for protein dynamics studies:*
   spin relaxation as a unique tool to study protein dynamics; overall and internal motions of a protein.
8. *Novel methods for structure determination*:
   orientational constraints from molecular alignment and anisotropic rotational diffusion.
9. *Computational aspects:*
   simulated annealing and molecular dynamics simulations.
10. *Protein structure prediction:* homology modeling.

Textbooks:

Required:

1. Cantor & Schimmer, Biophysical Chemistry, W.H.Freeman. (C&S)
2. Cavanagh et al., Protein NMR Spectroscopy. Academic Press, 1996 (Cav)

Recommended:

3. Tinoco et al., Physical Chemistry. 3rd Ed., Prentice Hall, 1995 (Tin)
4. M.Levitt, Spin Dynamics: basics of nuclear magnetic resonance, Wiley, 2001 (Mlev)
5. Branden & Tooze, Introduction to protein structure, 2nd Ed, Garland Publ. (B&T).

Grading:

Homework problems will be assigned regularly. Students will be given three major projects that will be due in class on the assigned dates. In addition, each student will be assigned a topic for oral presentation in class, on the last day of classes.
Contributions to the total grade:

Project #1 (X-ray)	20%
Project #2 (NMR)	20%
Project #3 (NMR)	20%
Topic presentation	20%
Final Exam	20%

A Detailed Syllabus

Date	Topic	Source	Comments
September			
3	Introduction to protein structure determination/Quiz	B&T	
5	Proteins as heteropolymers, amino acids as building blocks; interactions; backbone conformations, Ramachandran plots. The “sequence-structure” dogma.	B&T	HW #1
9	Proteins: protein architecture, hierarchy of levels of protein structure, motifs.	B&T	
13	Crystallography: X-ray diffraction, physical principles, von Laue conditions.	Tin. Ch 12	
16	Crystallography: Structure factor equation	Tin. Ch 12	HW #2
20	Crystallography: Symmetries and space groups	Tin. Ch 12	

23	Crystallography: Real vs reciprocal space.	Tin. Ch 12	
27	Crystallography: From crystal to structure, Phase problem.	Tin. Ch 12	HW #3
30	Practical crystallography: X-ray crystallography lab		Dr.Fettinger
October			
4	Crystallography: Phase problem (cont). Neutron, electron diffraction.	Tin. Ch 12	
7	NMR: General principles of NMR spectroscopy, classical description, Bloch's equations, chemical shift.	Cav, Ch.1	Project #1 (X-Ray) due
11	Quantum mechanical NMR Spectroscopy	Cav, Ch.2	
14	Product operator formalism. The rules.	Cav, Ch.2	
18	Examples of product operator calculations.	Cav, Ch.2	HW #4
21	Coherence selection, phase cycling, gradients.	Cav, Ch.4	VNMR
25	From 1-D to 2-D to n-D.	Cav, Ch.3,4	VNMR
28	Coherence transfer and mixing: COSY, NOESY, TOCSY.	Cav, Ch.4	HW #5
November			
1	Protein NMR for structure determination: NOEs, J-couplings, H-bonding.	Cav, Ch.6	
4	Homonuclear NMR approaches. Spin system assignment, NOESY signal assignment, example of structure assignment and calculation, 2^o structure prediction (J-coupling, Karplus eq).	Cav, Ch.6	
8	Experimental aspects: quadrature detection, sign discrimination. Data processing: window function, zero-filling, linear prediction. Computer simulations (VNMR)	Cav, Ch.3	VNMR
11	Heteronuclear coherence transfer: INEPT, HSQC, HMQC, TROSY.	Cav, Ch.4	VNMR
15	Triple-resonance methods: spin system typing, sequential assignment, 2^o structure prediction (CSI), isotope filtering/editing.	Cav, Ch.7	Project #2 (NMR) due
18	Novel methods for structure determination: orientation from residual dipolar couplings.	Handout notes	Dr. de Alba
22	Relaxation & Dynamics	Handout notes	
25	Novel methods for structure determination: orientation from relaxation anisotropy.	Handout notes	Project #3 (NMR) due
29	Modeling: Structure prediction	Handout notes	
December			
2	Homology modeling	Handout notes	
6	Modeling: MD simulations	Handout notes	HW #6
9	Modeling: MD simulations	Handout notes	
13	Assigned topics presentation		