BCHM669B Methods for Protein Structure Determination FALL 2004

Course meets in CBSO 2118, TuTh 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, studies of protein-ligand interactions, 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: the "Virtual NMR spectrometer".
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, anisotropic rotational diffusion, and CSA tensors.
9. *NMR methods to study protein-ligand interactions*.
   mapping protein-ligand interface, from fast to slow exchange, determining binding affinities.
10. *Computational aspects:*
    simulated annealing, molecular dynamics simulations, molecular docking.

10\. *Protein structure prediction:* homology modeling.

Textbooks:

<u>Required:</u>

1. Cantor & Schimmel, Biophysical Chemistry, W.H.Freeman. (C&S)
2. Van de Ven, Multidimensional NMR in Liquids, Wiley-VCH. (vdV)

<u>Recommended:</u>

3. Tinoco et al., Physical Chemistry. 3rd Ed., Prentice Hall, 1995 (Tin)
4. Cavanagh et al., Protein NMR Spectroscopy. Academic Press, 1996 (Cav)
5. M.Levitt, Spin Dynamics: basics of nuclear magnetic resonance, Wiley, 2001 (Mlev)
6. 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%