BIOC 6740 (Fall 2014) “Topics in Biophysics”

SYLLABUS

INSTRUCTOR: Dr. Jose L. Soulages  
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CLASSROOM AND CLASS HOURS

Classroom: 348B Noble Research Center  
Class Hours: TBA during first meeting

First meeting with students: 8/22/2014 at 2PM (348B NRC)

BIOC 6740 is a two-credit hour course directed to students pursuing a Master or Ph.D. degree in biochemistry. The course could be useful to students from other areas of the biological sciences. Students from the departments of microbiology and entomology and plant pathology have taken this class. The course covers spectroscopic concepts of general applicability to all spectroscopic techniques and treats in more detail experimental and theoretical UV-Visible absorption and fluorescence spectroscopy. Some years, depending on the students’ interest, BIOC 6740 also covers basic thermodynamic principles that are needed to understand physical chemical processes of biochemical interest, such as the energy transactions involved in metabolic pathways, the stability of proteins, the interaction between proteins and ligands, etc.

The course involves: Lectures, problems, quizzes and tests

OFFICE HOURS

The students are highly encouraged to visit with the instructor to discuss matters related to topics of the lectures and/or homework problems.

The instructor will arrange by e-mail consultation meetings at times convenient for the student.
TEXTS:
The instructor will provide copies of all slides used in lectures.
The spectroscopic concepts and information discussed in the course are well covered in two textbooks that are available online from the OSU library:

Modern Optical Spectroscopy. With Exercises and Examples from Biophysics and Biochemistry.
William Parson (2009) Publisher: Springer Berlin Heidelberg
Free online book from OSU library. Go to: http://www.springerlink.com/content/v15m76/

Lakowicz, Joseph R.
Free online book from OSU library. Go to: http://www.springerlink.com/content/q3003p/

If thermodynamic concepts are included in the course, the students will need to find or buy a book for the thermodynamic section of the course. Many books could be used. Among them:

Thermodynamics and Kinetics for the Biological Sciences
Gordon G Hammes
ISBN 0-471-37491-1
(This book is recommended but it only presents a simplified, oversimplified at times, overview of some key biophysical topics. The instructor will provide additional insights of the topics covered and, if needed, the student could consult other textbooks*).

* The two books indicated below provide a more in depth and detailed account of the thermodynamic topics:
Physical Chemistry for the Life Sciences/Peter Atkins and Julio de Paula/2006/ ISBN: 0-7167-8628-1


GRADING
The following activities will be graded:

1. Quizzes
2. Hour-exam
3. Final Comprehensive Exam

Grades: 100-80 = A; 79-65 = B; 65-50 = C; 49-40=D, and < 40 = F.

The final grade will be the higher of either: 1) the grade of the comprehensive final exam or 2) the grade calculated through the following addition:

10 % of the average grade obtained in quizzes
50 % of the average grade in hour exams
40 % of the grade achieved in the comprehensive final (***
Quizzes and/or hour exams that are not taken will be given a grade equal to that of the final comprehensive exam. If a student misses all quizzes and exam, the final grade for the course will be that of the final exam.

Note that responses to questions asked during lectures will not be evaluated. Students should feel free and are encouraged to ask or answer questions during lectures.

ATTENDANCE:
Attendance to lectures is not required but is highly recommended. Take into account that in previously assigned dates the students will be given quizzes.

The fall 2014 syllabus attachment provides important dates and offices available for different types of assistance. It can be found on the Academic Affairs' website:
http://academicaffairs.okstate.edu/faculty-a-staff

or at
http://academicaffairs.okstate.edu/images/Patty/FacultyandStaffResources/Syllabus/fall%202014%20syllabus-final.pdf

STUDENTS WITH DISABILITIES: If any student feels that he/she has a disability and needs special accommodations of any nature whatsoever, the instructors will work with you and the Office of Disabled Student Services, 326 Student Union, to provide reasonable accommodations to ensure that you have a fair opportunity to perform in this class. Please advise the instructor of such disability and the desired accommodations at some point before, during or immediately after the first scheduled class period.
Course Outline: BIOC 6740 (2014) Topics in Biological Spectroscopy

I. Review on Interaction of Radiation and Matter

Properties of Electromagnetic Waves

- Frequency, phase, polarization.
- Superposition of EM waves
- Plane, circular and elliptically polarized radiation.
- Coherence

Interaction of Electromagnetic Radiation with Matter

- The dipole oscillator
- Scattering, Reflection and Refraction
- Polarizability and refractive index
- Dispersion curves and birefringence.
- Methods to produce and analyze EM radiation of different energy in the UV visible range:
  - Lamps, prisms, diffraction gratings
- Production and analysis of polarized radiation.
- Modulation of light intensity

Absorption of Light

- Energy of Radiation and Transitions
- The Transition Dipole Moment and the Probability of Absorption
- The Direction of the Transition Dipole Moment
- Polarized Absorption Spectra
- Einstein Relationships for Absorption and Emission of Radiation
- Boltzmann's Distribution
- Lambert-Beer Law
- Lasers

II. UV-Visible Absorption Spectroscopy

Theory

- Rate of Electronic Transitions
- Frank-Condon Principle
- Spectrum Shape
- Linewidth: Natural and the uncertainty principle
- Effect of Intermolecular Interactions on Linewidth
- Effect of Intermolecular Interactions on the Energy of the Transition: Solvent-induced spectral shifts
- Transitions in the carbonyl and amide chromophores
- Interactions between chromophores: Band splitting and Changes in Intensity
Instrumental Aspects of UV-Visible Absorption spectroscopy

- Single Beam Spectrophotometers
- Split-Beam Spectrophotometers
- Dual wavelength Spectrophotometer
- Diode Array Spectrophotometer
- Resolution, accuracy and sensitivity of a spectrophotometric measurement
- Effect of stray light and Noise
- Comparison Different Spectrophotometers: Resolution, accuracy, precision, speed and sensitivity

Analytical Methods used in UV-Visible Absorption Spectroscopy

- Sample: preparation and stability
- Selection of cuvette
- Temperature control: effect on pH
- Single Wavelength determination of concentrations
- Multi-component samples and the use of Multiple wavelengths
- Isosbestic Points
- UV absorption Spectra of Proteins and chromophores of biological importance.
- Difference Spectra
- Derivative Spectroscopy.

III. Fluorescence Spectroscopy

1. **Principles:**
   - Jablonsky Diagram
   - Nonradiative Relaxation of Electronically Excited Molecules: Vibrational Relaxation and Internal Conversion.
   - Deactivation of the thermally-equilibrated lowest excited singlet state: radiative and nonradiative processes
   - Fluorescence
   - Singlet-triplet Intersystem Crossing
   - Phosphorescence
   - Delayed Fluorescence.
   - Rates of Different Processes of Deactivation
   - Stokes Shift
   - Fluorescence Lifetime
   - Quantum Yields

2. **Steady-State Fluorescence Spectroscopy**
   - The steady-state spectrofluorometer.
   - Excitation and emission spectra: Acquisition and Corrections
   - Steady State Fluorescence Intensity: Effects of: quantum yield, absorbance, intensity of exciting radiation, and slit widths
   - Relationship between fluorophore concentration and fluorescence intensity
   - Ideal Relationship, Inner Filter Effect
   - Experimental Considerations:
Concentration of Fluorophores and Chromophores
Scattering: Rayleigh and Raman
Selecting the Excitation Wavelength
Sensitivity of fluorescence: comparison with UV absorption techniques.

3. **Fluorescence in Proteins**
   Most common Fluorophores: Phe, Tyr and Trp
   Applications to study environment of fluorophores
   Position of Maximum and Quantum Yields
   Effect of pH, denaturants and Environment
   Green Fluorescent Proteins

4. **Fluorescence Quenching**
   - *Collisional Quenching*: The Stern-Volmer Equation.
   - Steady State and Lifetime Studies of Solute Quenching
   - *Static Quenching*
   - Applications of quenching studies: Study of dynamic and structural properties of macromolecules, ligand-binding and lipid-protein interaction.

5. **Fluorescence Polarization**
   - Definition of Anisotropy and Polarization Ratio.
   - Photoselection;
   - The emission transition dipole moment
   - Anisotropy of Florescence
   - Depolarization by Orientation Dependent Absorption;
   - Depolarization by non-co-linearity between absorption and emission dipoles
   - Depolarization by Molecular Motions
   - Time Dependent Anisotropy of Fluorescence
   - Steady State Anisotropy of Fluorescence
   - Perrin’s equation;
   - Macromolecular size and shape;
   - Study of chromophore dynamics in membranes and proteins;
   - Studies on Ligand-Protein and protein-protein association
   - Experimental Considerations: The G-factor

6. **Resonance Energy Transfer**
   - Forster Theory
   - The overlap integral
   - The $\kappa$ factor
   - Forster’s Distance
   - Applications: Measurements of intermolecular distances, protein-protein interaction, membrane-protein interactions, Ligand-binding, topology of membrane proteins, lipid-exchange between membranes and between proteins and membranes.
7. **Time-resolved Fluorescence Spectroscopy**
   - Why measuring a fluorescence lifetime?
   - Theory of Frequency-Domain fluorometry
   - Data Analysis
   - Principle of frequency-domain measurements
   - Instrumentation: Light modulator, cross-correlation, Frequency synthesizers and radio frequency amplifiers
   - Applications of time resolved measurements in FRET and in quenching studies
   - The measurement of Lifetimes-distributions using frequency-domain fluorometry

IV. **Some Biochemical Applications of UV-Absorption Spectroscopy**
   - Protein folding: Stability and kinetics of folding
   - Ligand-Binding: Experimental and theoretical approaches for single site and multiple equilibria
   - Cell Biology: GFPs, membrane and organelle probes
   - DNA melting
   - Chromophore Environment