MECHANISMS AND APPLICATIONS OF BIOLUMINESCENCE: PRACTICAL COURSE

Basic Information

This is a course, which contributes to MSc award in Biology

Course period	From February 1st till June 1st, 1 semester (18 weeks)
Study credits	6 ECTS credits
Duration	216 hours
Language of instruction	English
Academic requirements	– BSc degree in Biology, Physics, Biophysics, Chemistry , Biochemistry, Environmental Sciences or equivalent (transcript of records), – good command of English (certificate or other official document)

Course Description

The practical course "Mechanisms and applications of bioluminescence" is a part of International Master Program "Biological Engineering" offered by Siberian Federal University. It is designed to equip students with practical skills to perform different types of bioluminescence reactions and acquaints them with the principles of bioluminescent assays applied in environmental monitoring, medicine and other practical fields.

The course consists of three parts (modules). In the first module the biochemical basis of the most popular bioluminescent reactions are considered. The second module covers various methods of expression, purification and characterization of bioluminescent and fluorescent proteins and their application as BRET technique. The third module acquaints with the variety of bioassays based on enzymatic systems.

Special Features of the Course

The course is developed by one of the world's leading schools of scientific thought in the field of biological light-emitting systems and puts a strong emphasis on practical laboratory work.

Course Aims

The practicum provides an opportunity to master main techniques for measuring bioluminescence *in vitro* and learn to apply them in environmental monitoring, medical

diagnostics, pharmaceutical and food industries, etc. It aims to enable students to master the basic techniques for performing bioluminescence reactions *in vitro* and to apply bioluminescence assays in various fields.

Course Objectives

The course has been designed to:

- provide students with an opportunity to test various chemical bases of bioluminescent reactions and their kinetic properties;
- enable students to experience the whole set of methods starting from expression and purification of target recombinant proteins to their characterization and direct application;
- give students an understanding of principles of bioluminescent bioassay design.

Learning Outcomes of the Course

After completing the course students should be able to:

- experimentally implement different types of bioluminescent reactions and analyze their kinetic profiles;
- extract, purify and characterize various types of bioluminescent and fluorescent recombinant proteins;
- understand the principles of bioluminescent assays as applied in environmental monitoring, medical diagnostics, pharmaceutical and food industries, etc.

Course Outline

Week	Lectures	Practical work / Assignments	Hours
1-5	Module 1 «Mechanisms of bioluminescent reactions»		18
	• Responsive lecture «Introduction into bioluminescence chemistry» • Lecture «Introduction into laboratory techniques»	• Pre-course test • Self-Study Assignment 1.1 • Lab «Kinetics of the bioluminescent reaction catalyzed by bacterial luciferase» • Lab «Kinetics of the coupled enzyme bacterial bioluminescent reaction» • Lab «Kinetics of the firefly bioluminescent reaction» • Lab «Kinetics of the Ca^{2+}-dependent bioluminescent reactions»	

		• Home Assignments №№ 1.1-1.4	
6-13	Module 2 «Bioluminescent System of *Clytia gregaria*»		32
	• Lecture «Apo-clytin extraction from *E.coli* RIPL cells» • Lecture «Apo-clytin purification using ion-exchange chromatography under denaturing conditions» • Lecture «GFP extraction from *E.coli* XL1-Blue cells and its purification with affinity chromatography» • Lecture «Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)» • Lecture «Absorption spectra of various conformational states of clytin and GFP» • Lecture «Methods to determine protein concentration» • Lecture «Förster energy transfer (FRET) in the system clytin-cgreGFP: spectroscopic studies» • Lecture «Förster radius and energy transfer efficiency»	• Lab «Apo-clytin extraction from *E.coli* RIPL cells» • Lab «Apo-clytin purification using ion-exchange chromatography under denaturing conditions» • Lab «GFP extraction from *E.coli* XL1-Blue cells and its purification with affinity chromatography» • Lab «Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)» • Lab «Absorption spectra of various conformational states of clytin and GFP» • Lab «Methods to determine protein concentration» • Lab «Förster energy transfer (FRET) in the system clytin-cgreGFP: spectroscopic studies» • Lab «Förster radius and energy transfer efficiency» • Home Assignments 2.1-2.8	

	Module 3 «Bioluminescent Bioassays»		
14-18	• Lecture «Biological methods for environmental monitoring» • Lecture «Principle of bioluminescent enzymatic toxicity assays» • Lecture «How to design new enzymatic bioassays for toxicological analysis» • Lecture «Methods for stabilization of enzymes»	• Lab «Effect of pollutants on ADH and LDH activity» • Lab «Effect of pollutants on activity of coupled enzyme system NAD(P)H:FMN-oxidoreductase+luciferase» • Lab «Effect of food preservatives on trypsin activity» • Lab «Stability of soluble and immobilized enzymes to pH variation» • Self-Study Assignments №№3.1- 3.4 • Final Test	18

Lecturers and Tutors. Contact Information

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Assessment

The overall course percentage grade will consist of the final test results (20%) and the combined grades of 3 modules assignments (80%).

The score for each module is the sum of the following assignments:

- Module 1 (25% from 80%):
  - pre-course test on the chemistry of bioluminescence (5%),
  - reports on 4 lab works (each 5%, 20% total).

- Module 2 (35% from 80%):
  - lecture attendance (5%),
  - written answers to the 8 question sets (each 2.5%, 20% total),
  - instructor's evaluation of student's level of lab work participation and success (10%).

- Module 3 (20% from 80%):
  - lecture attendance (each 1%, 4% total),
  - written answers to 4 question sets (each 1%, 4% total),
  - reports on 4 lab works (each 3%, 12% total).

The overall course percentage grade will be converted into a letter grade as follows:
A = 91-100%
B = 81-90%
C = 71-80%
D = 61-70%
E = less than 61%.

Attendance Policy

Since almost every class contains practical work in laboratory, attendance of all of them is obligatory. Missing one or more of laboratory work classes will result in "No final grade".

Web page of the course

The webpage of the course *«Mechanisms and Applications of Bioluminescence: Practical Course»* is available through E-learning SibFU web site: www.e.sfu-kras.ru. You must be logged in to access this course. Course Guide and all accompanying materials are also available at the course web-page.

Core reading

Selected chapters of the books:

- Lee J. Bioluminescence, the Nature of the Light. – 2016.
- Shimomura O. Bioluminescence: Chemical Principles and Methods, Revised Edition. – 2012.
- Haddock S. H. D. Bioluminescence in the sea. / S. H. D. Haddock, M. A. Moline, J.F. Case. // Annu. Rev. Mar. Sci. 2010. 2, PP. 443-493.
- Lakowicz J. R. Energy Transfer. In Principles of Fluorescence Spectroscopy. 3rd edition. 2006. Springer. ISBN: 978-0-387-46312-4
- Nelson D. L. and Cox M. M. Lehninger Principles of Biochemistry. 5th edition.

Journal articles:

- Esimbekova E., Kondik A., Kratasyuk V. Bioluminescent enzymatic rapid assay of water integral toxicity. Environmental Monitoring & Assessment. Vol. 185 Issue 7, PP. 5909-5916. DOI: 10.1007/s10661-012-2994-1.
- Esimbekova E. N., Lonshakova-Mukina V. I., Bezrukikh A. E., Kratasyuk V. A. Design of multicomponent reagents for enzymatic assays. In: Doklady Biochemistry and Biophysics. Vol. 461 Issue 1, PP.102-105.

Electronic articles:

- Kratasyuk V. and Esimbekova E. Analytical applications of luminous bacteria enzymes. In Photobiological sciences online / John Lee, Kendric C. Smith, editors and webmasters. American Society for Photobiology. ISSN 2470-2749
- Branchini B. Chemistry of firefly bioluminescence. In Photobiological sciences online / John Lee, Kendric C. Smith, editors and webmasters. American Society for Photobiology. ISSN 2470-2749
- Lin L. Y.-C. and Meighen E. A. Bacterial bioluminescence: biochemistry and molecular biology. In Photobiological sciences online / John Lee, Kendric C. Smith, editors and webmasters. American Society for Photobiology. ISSN 2470-2749