REQUIREMENTS

Semmelweis University, Faculty of General Medicine – single, long-cycle medical training programme
Name of the host institution (and any contributing institutions): Department of Biochemistry

Name of the subject: Quantitative Biochemistry

Credit value: 2

Semester: 2.

Total number of classes per week:	**lectures: 1**	**practical lessons: 1**	**seminars:**

Type of subject: compulsory **optional** elective

Academic year: 2024/2025

Language of instruction, for optional or elective subjects: English

Course code: 830

(In the case of a new subject, this cell is filled in by the Dean's Office, following approval)

Course coordinator: Prof Krasimir Kolev
Place of work, phone number: Department of Biochemistry

H-1094 Budapest, Tűzoltó u. 37-47. tel: +36-1-459-1500#60010 email: Kolev.Krasimir@semmelweis.hu

Position: professor

Date and number of habilitation: 2008/266

Objectives of the course and its place in the medical curriculum:

The Quantitative Biochemistry course is recommended for students planning to be involved in biomedical research. It offers a concise overview of the cell's dynamic metabolic networks, focusing on the general principles of enzyme kinetics, structure, and control of metabolic pathways. Aspects that are essential for future researchers in any area of medicine are emphasized: modern biochemical techniques in the characterization of intermolecular interactions and enzyme action, and in silico modeling of biochemical processes and systems. Medical orientation is implemented with a discussion of examples of quantitative approaches in analyzing the molecular targets of therapy. Students participate in formal lectures, and practical lessons and prepare individual project work.

Place of instruction (address of lecture hall or seminar room etc.):

the premises of the Basic Medical Science Center, H-1094 Budapest, Tűzoltó u. 37-47

Competencies acquired through the completion of the course:

a) knowledge
-molecular mechanisms and modelling of biological processes
-molecular regulation of biochemical processes and their dysregulation in pathology
b) skills
-modelling and simulation of biochemical processes
-quantitative assessment of the reliability of biochemical models

Prerequisites for course registration and completion:

Completion (or concurrent registration also allowed) of the Medical Biochemistry 1 course in Hungarian (AOKBMT794_1M or AOKBMT10991M) or English (AOKBMT794_1A or AOKBMT10991A). The course is designed in line with the institutional strategy of Semmelweis University approved by the Senate with decree **#19/2021(IV.08)** to provide an institutional framework for the Hungarian students to acquire a part of their credits in English language.

Conditions for concurrent course registration and permission thereof in the case of a multi-semester subject: NA

Student headcount conditions for starting the course (minimum, maximum) and method of student selection:

Minimal: 10; maximal: 30. Students are selected based on grades in Medical Chemistry.

Detailed course description:

Lectures

Week 4 (Prof Kolev, 3 hours)
Introduction to metabolism. Metabolic pathways, kinetic and thermodynamic approaches to their structure. Enzyme-catalyzed reactions. Enzyme kinetic models. Briggs-Haldane steady-state approach and non-steady state systems. Coenzymes, cofactors.

Week 5 (Prof Kolev, 3 hours)
Enzyme activity assays. Methods for evaluation of enzyme action and its modulation. Spectrophotometric and fluorimetrc procedures. Zymography. Surface plasmon resonance. Microcalorimetry. Factors affecting the catalytic efficiency of enzymes.

Week 6 (Prof Kolev, 3 hours)
Kinetic parameters and their interpretation in the context of metabolic pathways. In vivo significance of kinetic parameters. Structural aspects of the control of enzyme activity. Allosteric and active site directed effects. Proteolytic activation and inactivation. Reversible covalent modifications.

Week 7 (Prof Kolev, 3 hours)
Basics of metabolic control analysis. Control, elasticity and response coefficients in metabolic pathways. Practical applications in drug research.

Week 8 (Prof Kolev, 3 hours)
Stochastic nature of experimentally determined parameters in biochemistry. Statistical error and probability distribution of estimates. Models and simulations in enzyme kinetics.

Seminars/practical classes

Week 9 (Prof Kolev, 3 hours)
In-person comprehension of instructions and guidelines for the individual project works

Week 10 (Prof Kolev, 3 hours)
Computer simulated enzyme kinetics 1. Continuous and end-point enzyme assays. Adjusting the conditions of the simulated experiment in accordance with the assumptions of the steady-state approach. Best experimental guess of the model parameters and their confidence intervals.

Week 11 (Prof Kolev, 3 hours)
Computer simulated enzyme kinetics 2. Application of enzyme kinetics for solution of practical problems. Identification of physiological substrates and high control-coefficient steps in metabolic pathways. Identification of amino acids with critical function in the catalytic mechanism of enzymes.

Week 12 (Prof Kolev, 3 hours)
Submission and defence of the project works

Related subjects due to interdisciplinary fields (both compulsory and elective) and potential overlaps between subjects:

Medical Biochemistry (no overlap).

Attendance requirements; conditions under which students can make up for absences and the method of absence justification:

Attendance in-person is mandatory. Up to 6 h (2 weeks) absences are tolerated, no justification is needed. No make-up for absences is possible.

Form of assessment in the study period:

The individual project work and its defence are graded. A minimal grade of 2 is a condition for the signature.

Number and type of assignments for individual work and the deadline for submission:

A single assignment (individual project work) to be opened in week 9 and submitted in week 12.

Requirements to obtain the teacher's signature:

Attendance of the classes (lectures and practical) with no more than 2 absences and successful project work submitted by the deadline.

Type of assessment:

comprehensive examination (written and oral)

Examination requirements:
The exam grade is the average of the graded individual project and the oral exam based on the topics below.
Examination topics in Quantitative Biochemistry

1. Significance of Gibbs' energy change in the metabolism
2. Reactions coupled in-series in the metabolism
3. Reactions coupled in-parallel in the metabolism
4. Enzyme kinetics and metabolism
5. Molecularity and order of reactions
6. Practical determination of the order of reactions
7. Determination of the standard activation enthalpy from reaction rate constants
8. Interpretation of the Gibbs' energy of activation
9. Macromolecular nature of the enzymes and conformational entropy
10. Enzyme effects on the activation enthalpy and entropy
11. The original Michaelis-Menten model of enzyme kinetics
12. The Briggs-Haldane steady state model of enzyme kinetics
13. The current form of the Michaelis-Menten equation and interpretation of its parameters
14. The hyperbolic plot of the Michaelis-Menten equation
15. Interpretation of the specificity constant

16. What is the proper measure of catalytic efficiency of isoenzymes?
17. Alternative plots of the Michaelis-Menten equation
18. Direct linear plot of the Michaelis-Menten equation
19. Kinetic parameters of two-substrate reactions
20. Reversible Michaelis-Menten mechanism
21. Three-step Michaelis-Menten mechanism
22. When is Km a measure of affinity?
23. „One-way" enzymes
24. Product inhibition
25. Tools for measuring enzyme activity (absorbance, fluorescence)
26. Tools for measuring intermolecular interactions (time-resolved fluorimetry, FRET, surface plasmon resonance, isotherm titration calorimetry)
27. The problem of initial rate
28. The problem of enzyme inactivation: Selwyn's test
29. Practical issues in the design of a kinetic measurement
30. Modelling the effects of temperature on enzyme activity
31. Modelling the effects of pH on enzyme activity (enzymes as dibasic acids)
32. The ionization state of the substrate and the pH dependence of the enzyme activity
33. Reversible inhibition of enzymes
34. Linear and hyperbolic inhibition of enzymes
35. Competitive inhibition
36. Uncompetitive inhibition
37. Mixed type inhibition
38. Relation of inhibition constants and IC50
39. Determination of inhibition constants
40. Modelling the combined action of several inhibitors
41. Modelling the the presence of two competing substrates
42. Distinct in vivo consequences of the specificity and catalytic component of inhibition
43. Irreversible inhibitors (serpins)
44. Distinct pharmacological consequences of various inhibitor effects
45. Experimental design for characterization of the inhibition
46. General modulator effects (mixed hyperbolic inhibition and activation)
47. Regulatory efficiency in Michaelis-Menten kinetics and linear inhibition
48. Allosteric effects in the regulation of enzymes
49. Simple and cooperative binding saturation curves
50. Saturation equation of cooperative binding
51. Hill equation
52. Cooperativity index
53. Symmetry model of cooperativity
54. Alternative cooperativity models (sequential model)
55. Complex regulation of glycogen phosphorylase (allosteric, cyclic covalent, double cyclic covalent).
56. One-way covalent modification of enzymes
57. Regulation of enzymes through specialized subunits
58. Signal amplification in the substrate cycles
59. Arrangement of enzyme-catalysed reactions in metabolic pathways
60. Steady-state of metabolic pathways
61. Practical applications of the kinetic parameters: identification of physiological substrates
62. Practical applications of the kinetic parameters: efficiency of flux regulation
63. Estimation of the equilibrium state from data on the mass action ratio
64. In vivo Vmax as an indicator of regulatory role of the enzyme
65. Ratio of in vivo substrate concentration and Km at regulated steps
66. What is the start point and the end point of a metabolic pathway? The concept of metabolic control analysis
67. Elasticity of enzymes in metabolic pathways. The relation of elasticity and kinetic models
68. Practical aspects of elasticity and kinetic parameters
69. Elasticity with allosteric enzymes
70. Flux control coefficient of enzymes in metabolic pathways
71. Flux control coefficients at various enzyme levels

72. The summation theorem in metabolic control analysis
73. The relation of elasticity and flux control coefficient: the connectivity theorem
74. Combined application of the summation and connectivity theorems
75. Interpretation of the response coefficient
76. Determination of the flux control coefficient: genetic manipulations, titration with a purified enzyme or with an inhibitor, using a response coefficient and elasticity
77. Options to increase sensitivity to regulators: catalytic amplification, signal amplification, biological integration
78. Comparison of kinetic parameter estimation from various plots of the Michaelis-Menten equation
79. Inclusion of an error term in the Michaelis-Menden equation
80. Variants of errors: uniform coefficient of variation, uniform standard deviation
81. Classical assumptions of the least square analysis of enzyme kinetic models
82. Non-parametric estimation of Michaelis-Menten parameters using direct linear plot
83. Weighed least square estimates of Michaelis-Menten parameters
84. Experimental variance and comparison of enzyme kinetic models
85. Residuals and their use in model discrimination of the estimation of kinetic parameters
86. Presentation of the results in enzyme kinetics: "to-do"s and "not-to-do"s in the presentation of model and experimental data.

Method and type of grading:

The exam grade is the average of the graded individual project and the oral exam based on the topics listed above.

List of course books, textbooks, study aids and literature facilitating the acquisition of knowledge to complete the course and included in the assessment, precisely indicating which requirement each item is related to (e.g., topic by topic) as well as a list of important technical and other applicable study aids:

Required reading

1. Athel Cornish-Bowden: Fundamentals of enzyme kinetics. Wiley-Blackwell, Weinheim, 2012
https://www.wiley.com/en-us/Fundamentals+of+Enzyme+Kinetics,+4th+Edition-p-9783527330744
2. Lecture slides posted in the Moodle system
3. Video recordings of the lectures posted in the Moodle system

Recommended literature

David Fell: Understanding the control of metabolism. Portland Press, London, 2003

Signature of habilitated instructor (course coordinator) announcing the course:

Signature of the director of the host institution:

Date of submission: