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stamp of the Doctoral School

DESCRIPTION OF TRAINING PROGRAMME
FOT THE DOCTORAL SCHOOL AT THE KAZIMIERZ WIELKI UNIVERSITY

INFORMATION ON COURSE	
Course	**Researcher's workshop I**
Type of classes	Specialist classes
Academic year	**2020/2021**
Field of science	natural sciences
Discipline of science	**biological sciences**
Class instructor	**prof. dr hab. Joanna Moraczewska**
Number of hours	**30**
Form of classes	**laboratory**
Pass rules	**credit with a grade**
Language of lecture	**English**
Framework learning outcomes (8 PRK)	• knows and understands to such an extent that is possible to revise existing paradigms – world heritage, including theoretical foundations, general issues and selected specific issues – specific to a scientific or artistic discipline • knows and understands the main trends in the development of the scientific or artistic disciplines covered in the curricula • knows and understands research methodology • is able to critically analyse and evaluate the results of scientific research, expertise and other creative work and their contribution to knowledge development • is able to use knowledge from different fields of science or art to creatively identify, formulate and innovatively solve complex problems or perform research tasks, in particular: o define the purpose and subject of scientific research, formulate a research hypothesis, o develop research methods, techniques and tools, and use them creatively, o draw conclusions on the basis of scientific research • is ready for critical evaluation of the achievements of a given scientific or artistic discipline
DETAILED DESCRIPTION OF CLASSES	
Particular learning outcomes	• knows and understands current knowledge on structure and functions of actin cytoskeleton, molecular structure of different types of muscle cells, regulation of dynamics of actin filaments in muscle and non-muscle cells; • knows the unresolved problems in the studies of actin cytoskeleton regulation; • knows and applies standard techniques used for muscle proteins preparation;

	• knows and applies recombinant DNA techniques – cDNA synthesis, cloning, site-directed mutagenesis, DNA sequencing; • knows and applies techniques of protein expression in prokaryotic systems; • knows and is able to assemble chromatography instruments - low pressure and FPLC; • knows and is able to select resins for protein purification with the use of size-exclusion, ion exchange and affinity chromatography; • knows and applies methods of protein visualization by SDS-PAGE, native electrophoresis, Western Blot; • knows and is able to select appropriate techniques to quantify proteins, analyze association and dissociation constants; • understands and performs experiments on actin polymerization using steady-state fluorescence and light scattering techniques • proposes hypotheses and suggests experiments to verify protein-protein inter-actions; • based on the results of experiments draws conclusions on proteins interactions and effects of mutations on the interactions; • evaluates the obtained results, compares them with literature data and critically explains reasons of possible differences and discrepancies .

Program content implemented during classes

1. Preparation of skeletal muscle acetone powder, extraction and purification of actin.

2. Oligonucleotide-directed mutagenesis of tropomyosin cDNA – primer design, PCR, production of DNA in bacterial cells, mutant screening.

3. Expression of wild type and mutant tropomyosin.

4. Protein purification using chromatographic techniques.

5. Analysis of the effects of mutations on tropomyosin interactions with actin, determination of dissociation and association constants.

6. Analysis of the effects of mutation in tropomyosin on inhibition of actin polymerization by tropomodulin.

Didactic methods	Experiments on biological material with the use of scientific in-struments
Assessment methods and cri-teria	1. Evaluation of completeness of the laboratory logbook and the quality of descriptions of performed experiments. 2. Colloquium - oral assessment of the knowledge acquired on the techniques of protein analyses. 3. The final grade is the arithmetic mean of the partial grades for the logbook evaluation and colloquium.
Passing rules	Minimum required to pass – 60% 60-67% - 3.0 68-75% - 3.5 76-83 % - 4.0 84-91% - 4.5 above 91% - 5.0

Basic literature	M. Green and J. Sambrook *(2012) Molecular Cloning: A Laboratory Manual* (Fourth Edition). Cold Spring Harbor Laboratory Principles and techniques in biochemistry and molecular biology, Wilson K. Walker J., ed., Oxford University Press, 2005. J.R. Lakowicz, Principles of Fluorescence Spectroscopy, Kluwer Academic/Plenum Publishers, New York, 1999.
Supplementary literature	Moraczewska J, Robaszkiewicz K, Śliwinska M, Czajkowska M, Ly T, Kostyukova A, Wen H, Zheng W. (2019) Congenital myopathy-related mutations in tropomyosin disrupt regulatory function through altered actin affinity and tropomodulin binding. *FEBS J*. 286(10):1877-1893. Śliwinska M, Robaszkiewicz K, Czajkowska M, Zheng W, Moraczewska J. (2018) Functional effects of substitutions I92T and V95A in actin-binding period 3 of tropomyosin. *Biochim Biophys Acta. – Prot. Proteom.* 1866 (4): 558-568. Ostrowska Z, Robaszkiewicz K, Moraczewska J. (2017) Regulation of actin filament turnover by cofilin-1 and cytoplasmic tropomyosin isoforms. *Biochim Biophys Acta – Prot. Proteom.* 1865: 88-98. Robaszkiewicz K, Ostrowska Z, Marchlewicz K, Moraczewska J. (2016) Tropomyosin isoforms differentially modulate the regulation of actin filament polymerization and depolymerization by cofilins. *FEBS J.* 283(4):723-737.

ATTACHMENT FOR DESCRIPTION OF TRAINING PROGRAMME

Course	**Researcher's workshop I**
Forme of classes	stationary ~~/ manual/ mixed model~~*
Methods and techniques of distance learning	Literature studies Remote consultations
Form and date of individual consultations *	Mixed model - meetings in the laboratory and/or on Skype/MS Teams
Form of passing of assessment / examination	1. oral / ~~written~~ 2.manual / stationary 2. keeping laboratory logbook