

# DEPARTMENT OF BIOTECHNOLOGY

## B-3

*At the Department of Biotechnology we investigate the biological molecules of microbiological, fungal, plant and animal origin using modern biotechnological methods. We would like to apply them for diagnostic and therapeutic purposes in human and veterinary medicine, for plant protection, the preparation of quality and safe food and for the protection of the environment, contributing to an improvement of peoples' health and of the environment in which we live. Our research work is focused on the processes of cancer progression and immune response, neurodegenerative processes, the biology of fungi, plant stress response and in the search for new biotechnological approaches and products.*

As in previous years, also in 2016, we studied the structure and function of various fungal enzymes, inhibitors and lectins. Mushrooms represent a promising source of bioactive proteins and biopesticides based on their own arsenal of various compounds used against pests, parasites and pathogens. Among them, defense proteins are very important and include lectins and protease inhibitors with strong and specific insecticidal and/or nematocidal activity. Phylogenetic analyses revealed that these proteins are widely present among higher fungi, ascomycetes and basidiomycetes. These protein toxins have potential applications in veterinary and human medicine and in crop protection. We have also described the medicinal properties of mushrooms belonging to the genus of funnel mushrooms (*Clitocybe* sp.) and of lectins from clouded funnel (*Clitocybe nebularis*), which show antitumor, immunomodulatory, antioxidative and antimicrobial (antiviral, antibacterial, and antifungal) activities.

The search for novel antibacterial compounds from mushrooms in cooperation with the National Institute of Biology using the plant pathogenic bacterium *Ralstonia solanacearum* as the model led to isolation and characterization of the protein complex with L-amino acid oxidase activity. Interestingly, these enzymes with antibacterial activity that were previously described mainly as major components of snake venoms, are also present in poisonous mushrooms such as the death cap (*Amanita phalloides*) and surprisingly also in edible mushrooms such as the trooping funnel (*Clitocybe geotropa*).

The continuing study on the involvement of proteases in plant response to drought showed a new level of complexity demonstrating that different stages of water deficit correlate to different levels of peptidase activities in leaves of different ages in common bean plants.

The studies in the field of glycobiology in 2016 focused on lectins from different mushrooms and their effects on different cell lines, with emphasis on immune cells. We confirmed the exclusive cytotoxicity of CNL lectin from clouded funnel against leukemic T cells Jurkat causing programmed cell death through binding a receptor in a plasma membrane.

Investigating the role of proteolytic enzymes in the regulation of cell cytotoxicity we focused our work on cathepsins C and H, two main convertases of progranzyme B, the molecule which triggers the processes of cell death. The activity of cathepsins C and H in secretory vesicles is controlled by the endogenous inhibitor cystatin F. Using different cystatin F mutants we analyzed the mechanism of its secretion, activation and cell uptake on different cell lines. With different cystatin F mutants we identified its target proteases involved in the regulation of the cytotoxicity of natural killer cells (NK cells) and confirmed its involvement in the reduced cytotoxicity of anergized NK cells. To study the involvement of cystatin F in the regulation of cytotoxic T lymphocyte function we established a model of cytotoxic T lymphocytes with reduced cytotoxicity. Using this model we can further study mechanisms leading to lower cytotoxicity after the interaction of cytotoxic cells with tumor cells. We found increased cystatin F levels in cells with lower cytotoxicity. Furthermore, using antibodies against LAMP1 and flow cytometric analysis we confirmed that exocytosis is not blocked in these cells.

In the field of neurobiology we published three articles in reputable journals on the topic of molecular processes underlying frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS). In our most acclaimed publication and in collaboration with a large international consortium



Head:  
**Prof. Janko Kos**

- **New genes associated with amyotrophic lateral sclerosis were discovered**
- **The aggregation of TDP-43 leads to the loss of its function**
- **The current understanding of the role of nuclear transport in ALS and FTD was critically reviewed**



*Figure 1: Death cap is a source of various bioactive compounds including antibiotics and trypsin inhibitors.*

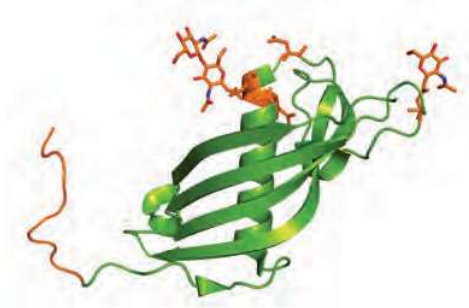


Figure 2: Ribbon diagram of human cystatin F with mutants indicated in orange.

- **L-amino-acid oxidases from Amanita phalloides and Clitocybe geotropa induce caspase-dependent apoptosis**
- **The functional recombinant B subunit of shiga toxin, Stx1B was produced and expressed on the surface of lactic acid bacterium Lactococcus lactis**

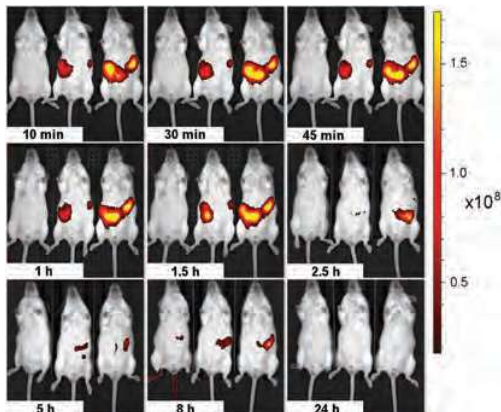
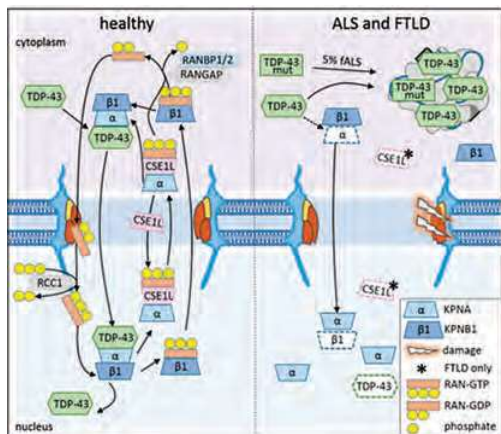


Figure 3: Representative example of 24h time-course imaging of mice administered with no bacteria (left; control),  $2.5 \times 10^{10}$  cells of IRFP713-expressing *L. lactis* (middle) and  $5.0 \times 10^{10}$  cells of IRFP713-expressing *L. lactis* (right). Colour bar indicates radiant efficiency.



we reported new genes and gene regions that are associated with the development of ALS. The study was published in the journal Nature Genetics and has received a substantial response in foreign and domestic media. Upon publication, we had several television, radio and newspaper interviews and reports. In the second article, we focused on the impact of the lack of protein TDP-43 on cell proteome (Prpar Mihevc et al., Scientific Reports). In cooperation with a group of ICGEB, Trieste, we found that the aggregation of TDP-43 leads to a similar lack of specific proteins as silencing or loss of TDP-43. We also published a review article in the prestigious journal Brain, on the nuclear transport in ALS (Prpar Mihevc et al., Brain).

Infections with shiga toxin-producing bacteria, like enterohemorrhagic *Escherichia coli* and *Shigella dysenteriae*, represent a serious medical problem. No specific and effective treatment is available for patients with these infections, creating a need for the development of new therapies. We have produced functional recombinant B subunit of shiga toxin, Stx1B, in *Escherichia coli* and used it as a target for the selection of ABD variants from the ABD scaffold-derived high-complex combinatorial library in collaboration with our Czech partners. We have selected 17 different ABD variants (named S1B) with a five-round ribosome display. The two most promising S1Bs (S1B22 and S1B26) were characterized into more details by ELISA, surface plasmon resonance and microscale thermophoresis. The binding affinity to Stx1B was determined to be in the  $1\text{-}\mu\text{M}$  range with both methods. The addition of S1Bs changed the subcellular distribution of Stx1B in HeLa cells, completely eliminating it from Golgi apparatus, most likely by interfering with its retrograde transport. All S1Bs were successfully displayed on the surface of safe lactic acid bacterium *Lactococcus lactis* by fusing to the Usp45 secretion signal and to the peptidoglycan-binding C terminus of AcmA. Binding of Stx1B by engineered lactococcal cells was confirmed using flow cytometry and whole-cell ELISA. Engineered lactic acid bacteria are potentially useful for the removal of Shiga toxin from human intestine. Additionally, novel ABD scaffold-derived Stx1B binders are useful for basic research on Shiga toxin-related infections and could be further improved for in-vitro diagnostics.

The results of the research work at the Department of Biotechnology in the year 2016 were published in 24 scientific papers in journals with an impact factor and in one book chapter. Also, three patent applications were filed. We received two research grants and Ph.D. grant from the Slovenian Research Agency, two bilateral international grants and as partners EU project 'Carbohydrate Metrology', CSA - Coordination & Support Action, Horizon 2020. Two Ph.D. students defended their doctoral theses. Ph.D. Young researcher Ana Bajc Česnik ended a one-year visit to the laboratory of Prof. dr. Cleveland, UCSD, USA. Head of department prof. Janko Kos was elected as a member of European Academy of Sciences and Arts. The members of the department were also very active in pedagogical work as lecturers and mentors to students preparing diploma and doctoral thesis at universities in Slovenia and abroad.

### Some outstanding publications in the past year

1. Prpar Mihevc, Sonja, Darovic, Simona, Kovanda, Anja, Bajc Česnik, Ana, Župunski, Vera, Rogelj, Boris. Nuclear trafficking in amyotrophic lateral sclerosis and fronto-temporal lobar degeneration. *Brain*, ISSN 0006-8950, [in press] 2016, 14 str., doi: 10.1093/brain/aww197. COBISS.SI-ID 29663527, IF 10,1.
2. Rheenen, Wouter Van, Shatunov, Aleksey, Dekker, Annelot M., McLaughlin, Russell L., Diekstra, Frank P., Pulit, Sara L., Van Der Spek, Rick A. A., Vösa, Urmo, De Jong, Simone, Robinson, Matthew R., Rogelj, Boris, Vrabec, Katarina, Ravnik-Glavac, Metka, Koritnik, Blaž, Zidar, Janez, Leonardis, Lea, Dolenc-Grošelj, Leja, et al. Genome-wide association analyses identify new risk variants and the genetic architecture of amyotrophic lateral sclerosis. *Nature genetics*, vol. 48, no. 9, str. 1043-1048. [COBISS.SI-ID 3106220], IF 31,6

Figure 4. Model of nuclear transport of TDP-43 in healthy individuals, and in cases of ALS-TDP and FTD-TDP. TDP-43 is translocated into the nucleus by the classical import pathway. In cases of ALS-TDP and FTD-TDP TDP-43 is mislocalized to cytoplasmic inclusions. Reduced levels of CSE1L in FTD-TDP and loss of nuclear KPNA1 in ALS-TDP imply that this is due to nuclear transport defect. Proteins with reduced levels are in dashed brackets. From Prpar Mihevc et al., *Brain* 2016).

- McKinnon, Brett D., Kocbek, Vida, Nirgianakis, Kostantinos, Bersinger, Nick A., Mueller, Michael D. Kinase signalling pathways in endometriosis : potential targets for non-hormonal therapeutics. Human reproduction update, ISSN 1355-4786, [in press] 2016, 22 str., doi: 10.1093/humupd/dmv060. [COBISS.SI-ID 29436455], IF 11,2

### Organization of conferences and meetings

- 10<sup>th</sup> Young researchers' day (Chemistry, Materials, Biochemistry, Environment), Jožef Stefan Institute, Ljubljana, 31 March 2016
- Day of Biomolecular Sciences – Biomolekularec 2016, Faculty of Medicine, University of Ljubljana, Ljubljana, 22 September 2016
- Annual meeting of co-workers of the research programme Pharmaceutical Biotechnology: Knowledge for Health, from the Department of Biotechnology at the Jožef Stefan Institute and the, Chair of Pharmaceutical Biology, Faculty of Pharmacy, University of Ljubljana, 1 December 2016

### Patent granted

- Tadej Rejc, Uroš Petrič, Jana Debeljak, Toni Bremec, Polonca Ferk, Mojca Lunder, Irena Roškar, Borut Štrukelj, Samo Kreft, Mixture of natural polyphenols from white fir wood for reducing postprandial glucose concentration, SI24984 (A), Slovenian Intellectual Property Office, 30. 11. 2016.

## INTERNATIONAL PROJECTS

- Functional and Structural Studies of Lectins from Mushrooms  
Dr. Jerica Sabotič  
Slovenian Research Agency
- The Role of C/EBP Alpha in Regulation of Cystatin F Expression  
Prof. Janko Kos  
Slovenian Research Agency

## RESEARCH PROGRAM

- Pharmaceutical Biotechnology: Knowledge for Health  
Prof. Janko Kos

## R & D GRANTS AND CONTRACTS

- Dysregulation of TDP-43 expression in amyotrophic lateral sclerosis and frontotemporal lobar degeneration  
Prof. Boris Rogelj
- Nitroxoline and its derivatives as new antitumour drugs  
Dr. Janko Kos
- Post-transcriptional regulatory networks in neurodegenerative diseases  
Prof. Boris Rogelj
- Genetics and pharmacogenomics of inflammatory bowel diseases and genetically related chronic immune diseases  
Prof. Boris Rogelj
- Pathogenic mechanism of the C9orf72 expanded hexanucleotide repeat mutation in neurodegeneration  
Prof. Boris Rogelj
- The role of cysteine protease inhibitors in NK cell mediated lysis of tumour cells  
Prof. Janko Kos
- Protein engineering of recombinant probiotic lactic acid bacteria for treatment of irritative bowel disease  
Prof. Borut Štrukelj

## VISITORS FROM ABROAD

- Dr. Tibor Hortobagy, University of Debrecen, Debrecen, Hungary, 24 May – 25 May 2016
- Prof. Daniel H. Wreschner, University of Tel Aviv, Tel Aviv, Israel, 4 July 2016
- Dr. Adeleta Softić, Faculty of Pharmacy, University of Tuzla, Tuzla, Bosnia and Herzegovina, 21 August – 31 August 2016
- Esmeralda Dautović, M.Sc., Faculty of Pharmacy, University of Tuzla, Tuzla, Bosnia and Herzegovina, 21 August – 22 November 2016

## STAFF

### Researchers

- Asst. Prof. Aleš Berlec
  - Prof. Janko Kos\***, Head
  - Prof. Boris Rogelj
  - Dr. Jerica Sabotič
  - Prof. Borut Štrukelj\*
- Postdoctoral associates
- Dr. Vida Kocbek
  - Dr. Špela Konjar
  - Dr. Anja Kovanda
  - Dr. Milica Perišić Nanut
  - Dr. Sonja Prpar Mihevc
  - Dr. Anja Pucer Janež

- Dr. Petra Zadravec, left 01.08.16

### Postgraduates

- Ana Bajc Česnik, B. Sc.
  - Dr. Simona Darović, left 01.06.16
  - Mirjana Malnar, B. Sc.
  - Mateja Prunk, B. Sc.
  - Katja Škrlec, B. Sc.
- Technical and administrative staff**
- Maja Šimaga, M. Sc.
  - Darja Žunič Kolar, retired 01.06.16

### Note:

\* part-time JSI member

# BIBLIOGRAPHY

## ORIGINAL ARTICLE

1. Maruška Budič, Blaž Cigič, Maja Šoštarič, Jerica Sabotič, Vladimir Meglič, Janko Kos, Marjetka Kidrič, "The response of aminopeptidases of *Phaseolus vulgaris* to drought depends on the developmental stage of the leaves", *Plant physiol. biochem. (Paris)*, vol. 109, pp. 326-336, 2016.
2. Jana Debeljak, Polonca Ferk, Miro Čokolič, Andrej Zavrtnik, Eva Tavčar Benkovič, Samo Kreft, Borut Štrukelj, "Randomised, double blind, crossover, placebo and active controlled human pharmacodynamic study on the influence of silver fir wood extract (Belinal) on post-prandial glycemic response", *Pharmazie*, vol. 71, no. 10, pp. 566-569, 2016.
3. Gorazd Drevenšek, Mojca Lunder, Eva Tavčar Benkovič, Borut Štrukelj, Samo Kreft, "Cardioprotective effects of silver fir (*Abies alba*) extract in ischemic-reperfused isolated rat hearts", *Food & nutrition research*, vol. 60, pp. 1-7, oct, 2016.
4. Jana Erjavec, Maja Ravnikar, Jože Brzin, Tine Grebenc, Andrej Blejec, Mateja Želko-Gosak, Jerica Sabotič, Janko Kos, Tanja Dreo, "Antibacterial activity of wild mushroom extracts on bacterial wilt pathogen *Ralstonia solanacearum*", *Plant dis.*, vol. 100, iss. 2, pp. 453-464, 2016.
5. Giovanni Grandi, Michael D. Mueller, Andrea Papadia, Vida Kocbek, Nick A. Bersinger, Felice Petraglia, Angelo Cagnacci, Brett D. McKinnon, "Inflammation influences steroid hormone receptors targeted by progestins in endometrial stromal cells from women with endometriosis", *J. reprod. immunol.*, vol. 117, pp. 30-38, 2016.
6. Vida Kocbek, Giovanni Grandi, Fabian Blank, Carlos Wotzkow, Nick A. Bersinger, Michael D. Mueller, Satoru Kyo, Brett D. McKinnon, "TNF $\alpha$ -induced IKK $\beta$  complex activation influences epithelial, but not stromal cell survival in endometriosis", *Mol. hum. reprod.*, vol. 22, no. 11, pp. 768-777, 2016.
7. Urban Košak, Boris Brus, Damijan Knez, Roman Šink, Simon Žakelj, Jurij Trontelj, Anja Pišlar, Jasna Šlenc, Martina Gobec, Marko Živin, Larisa Tratnjek, Martina Perše, Kinga Sałat, Adrian Podkowa, Barbara Filippek, Florian Nachon, Xavier Brazzolotto, Anna Więckowska, Barbara Malawska, Jure Stojan, Irena Mlinarič-Raščan, Janko Kos, Nicolas Coquelle, Jacques-Philippe Colletier, Stanislav Gobec, "Development of an in-vivo active reversible butyrylcholinesterase inhibitor", *Scientific reports*, vol. 6, pp. 1-16, Dec, 2016.
8. Jernej Luzar, Peter Molek, Mira Šilar, Peter Korošec, Mitja Košnik, Borut Štrukelj, Mojca Lunder, "Identification and characterization of major cat allergen Fel d 1 mimotopes on filamentous phage carriers", *Mol. immunol.*, vol. 71, pp. 176-183, Mar, 2016.
9. Brett D. McKinnon, Vida Kocbek, Kostantinos Nirgianakis, Nick A. Bersinger, Michael D. Mueller, "Kinase signalling pathways in endometriosis: potential targets for non-hormonal therapeutics", *Hum. reprod. updat.*, vol. 22, no. 3, pp. 382-403, 2016.
10. Ana Mitrovič, Jakob Kljun, Izidor Sosič, Stanislav Gobec, Iztok Turel, Janko Kos, "Cloquinol-ruthenium complex impairs tumour cell invasion by inhibiting cathepsin B activity", *Dalton trans. (2003)*, vol. 45, iss. 42, pp. 16913-16921, 2016.
11. Ana Mitrovič, Bojana Mirkovič, Izidor Sosič, Stanislav Gobec, Janko Kos, "Inhibition of endopeptidase and exopeptidase activity of cathepsin B impairs extracellular matrix degradation and tumour invasion", *Biol Chem*, vol. 397, issue 2, pp.165-174, 2016.
12. Anja Pišlar, Jerica Sabotič, Jasna Šlenc, Jože Brzin, Janko Kos, "Cytotoxic L-amino-acid oxidases from *Amanita phalloides* and *Clitocybe geotropa* induce caspase-dependent apoptosis", *Cell death discovery*, vol. 7, iss. 2, 2016.
13. Sonja Prpar Mihevc, Marco Baralle, Emanuele Buratti, Boris Rogelj, "TDP-43 aggregation mirrors TDP-43 knockdown, affecting the expression levels of a common set of proteins", *Scientific reports*, vol. 6, pp. 33996-1-33996-9, 2016.
14. Wouter van Rheenen et al. (177 authors), "Genome-wide association analyses identify new risk variants and the genetic architecture of amyotrophic lateral sclerosis", *Nat Genet*, vol. 48, no. 9, pp. 1043-1048, Sept, 2016.
15. Petra Zadravec, Lucie Marečková, Hana Petroková, Vesna Hodnik, Milica Perišić, Gregor Anderluh, Borut Štrukelj, Petr Malý, Aleš Berlec, "Development of recombinant *Lactococcus lactis* displaying albumin-binding domain variants against Shiga toxin 1 B subunit", *PLoS one*, vol. 11, no. 9, pp. 0162625-1-0162625-21, 2016.

## REVIEW ARTICLE

1. Jernej Luzar, Borut Štrukelj, Mojca Lunder, "Phage display peptide libraries in molecular allergology: from epitope mapping to mimotope-based immunotherapy", *Allergy (Cph)*, vol. 71, iss. 11, pp. 1526-1532, 2016.
2. Jure Pohleven, Janko Kos, Jerica Sabotič, "Medicinal properties of the genus *Clitocybe* and of lectins from the clouded funnel cap mushroom, *C. nebularis* (Agaricomycetes): a review", *Int. j. medic. mushrooms*, vol. 18, no. 11, pp. 965-975, 2106.
3. Sonja Prpar Mihevc, Simona Darovic, Anja Kovanda, Ana Bajc Česnik, Vera Župunski, Boris Rogelj, "Nuclear trafficking in amyotrophic lateral sclerosis and frontotemporal lobar degeneration", *Brain*, vol. 140, issue 1, pp. 13-26, 2016.
4. Jerica Sabotič, Robin Ohm, Markus Künzler, "Entomotoxic and nematotoxic lectins and protease inhibitors from fungal fruiting bodies", *Appl. microbiol. biotechnol.*, vol. 100, no. 1, pp. 91-111, 2016.
5. Borut Štrukelj, "Potencialni učinki kanabinoidov", *Farm. vestn. (Tisk. izd.)*, vol. 67, no. 2-3, pp. 69-73, 2016.
6. Miha Vodnik, Borut Štrukelj, Mojca Lunder, "Ghrelin receptor ligands reaching clinical trials: from peptides to peptidomimetics; from agonists to antagonists", *Horm. Metab. Res.*, vol. 48, no. 1, pp. 1-15, 2016.

## SHORT ARTICLE

1. Irena Roškar, Borut Štrukelj, Mojca Lunder, "Screening of phenolic compounds reveals inhibitory activity of nordihydroguaiaretic acid against three enzymes involved in the regulation of blood glucose level", *Plant foods hum. nutr. (Dordrecht)*, vol. 71, iss. 1, pp. 88-89, 2016.

## PUBLISHED CONFERENCE CONTRIBUTION

1. Jelena M. Janjic, Aleš Berlec, Christina Bagia, Lu S. Liu, Irenej Jerič, Michael Gach, Bratislav M. Janjic, Borut Štrukelj, "NIR and MR imaging supported hydrogel based delivery system for anti-TNF alpha probiotic therapy of IBD", In: *Reporters, Markers, Dyes, Nanoparticles, and Molecular Probes for Biomedical Applications VIII: 15-16 February 2016, San Francisco, California, United States*, (Proceedings of SPIE, vol. 9723), (Progress in biomedical optics and imaging, vol. 17, no. 35), pp. 972309-1-972309-10.
2. Maja Zakošek, Janko Mrkun, Breda Jakovac-Strajn, Katarina Pavšič Vrtač, Janko Kos, Anja Pišlar, Alenka Nemeč Svete, Petra Zrimšek, "Biomarkerji v semenski plazmi kot faktorji napovedi kakovosti kratkotrajno hranjenega merjaščevega semena", In: *6. Slovenski veterinarski kongres 2016: Portorož, 2. -3. December 2016*, (Slovenian veterinary research, Vol. 53, suppl. 17, 2016), pp. 106-109.

## INDEPENDENT COMPONENT PART OR A CHAPTER IN A MONOGRAPH

1. Jerica Sabotič, Janko Kos, "Fungal protease inhibitors", In: *Fungal metabolites*, (Reference Series in Phytochemistry), J. M. Merillon, ed., K. G. Ramawat, ed., Berlin, Springer, 2016, 33 pp.

## PATENT

1. Tadej Rejc, Uroš Petrič, Jana Debeljak, Toni Bremec, Polonca Ferk, Mojca Lunder, Irena Roškar, Borut Štrukelj, Samo Kreft, *Mixture of natural polyphenols from white fir wood for reducing postprandial glucose concentration*, SI24984 (A), Urad RS za intelektualno lastnino, 30. 11. 2016.

## MENTORING

1. Simona Darovic, *Modifications of FUS and implications in pathological changes in neurodegenerative diseases*: doctoral dissertation, Ljubljana, 2016 (mentor Boris Rogelj).
2. Petra Zadravec, *Development of genetically modified lactic acid bacteria with shiga toxin binding ability*: doctoral dissertation, Ljubljana, 2016 (mentor Aleš Berlec).

3. Valter Bergant, *Characterization of heterogeneous nuclear ribonucleoprotein H in cytoplasmic stress granules*: master's thesis, Ljubljana, 2016 (mentor Boris Rogelj).
4. Melisa Fazlić, *The function and localization of gamma-enolase in colon cancer Caco-2 cell line*: master's thesis, Ljubljana, 2016 (mentor Janko Kos; co-mentor Tjaša Vižin).
5. Tanja Jakoš, *Novel inhibitors of cysteine peptidase cathepsin X*: master's thesis, Ljubljana, 2016 (mentor Janko Kos; co-mentor Urša Pečar Fonovič).
6. Božena Kesić, *Isolation and characterization of cystatin F mutants*: master's thesis, Ljubljana, 2016 (mentor Janko Kos; co-mentor Milica Perišić Nanut).
7. Sabina Kolar, *Differentiation of human pluripotent stem cells is accompanied with LIN28A protein nuclear accumulation*: master's thesis, Ljubljana, 2016 (mentor Boris Rogelj).
8. Jure Loboda, *Protein and morphological analysis of vesicles secreted by human microglia*: master's thesis, Ljubljana, 2016 (mentor Janko Kos; co-mentor Metka Lenassi).
9. Mirjana Malnar, *Characterisation of L1 ORF1p in mammalian cells*: master's thesis, Ljubljana, 2016 (mentor Boris Rogelj).
10. Polona Megušar, *Characterisation of antimicrobial and antiadhesive activity of mushrooms aqueous extracts*: master's thesis, Ljubljana, 2016 (mentor Anja Klančnik; co-mentor Jerica Sabotič).
11. Klara Tereza Novoselc, *Expression changes in selected proteins after muscle inactivity*: master's thesis, Ljubljana, 2016 (mentor Boris Rogelj; co-mentor Anja Kovanda).
12. Maja Oven, *Evaluation of L-amino acid oxidases as inducers of apoptosis*: master's thesis, Ljubljana, 2016 (mentor Janko Kos; co-mentor Anja Pišlar).