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, 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, biology of fungi, plant stress response and in the search for new biotechnological approaches and products.

Head:
Prof. Janko Kos

Prof. Borut Štrukelj was a recipient of a Zois Award for outstanding scientific achievements

As in previous years, in 2015 we studied the structure and function of various fungal enzymes, inhibitors and lectins. With regard to structural similarities we investigated the interactions between the inhibitors and lectins, and demonstrated that they represent an important mode of molecular recognition being relevant under physiological conditions. In cooperation with the National Institute of Biology we have shown the insecticidal activity of clitocypin, the cysteine protease inhibitor from clouded agaric, against Colorado potato beetle larvae. As shown previously for macrocypins, cysteine protease inhibitors from parasol mushroom, clitocypin also inhibits digestive cysteine proteases, intestains, and it does not elicit the adaptive response in larval guts. This establishes mushrooms as an attractive source for novel biopesticides.

We continued our research of the enzyme L-aminooxidase (LAO) from Amanita phalloides and Clitocybe geotropa. Both enzymes, ApLAO and CgLAO, reveal a cytotoxic action on human T lymphocytes, activating apoptosis through the activation of caspase pathways. We showed that the intrinsic pathway is a predominant one. The results could be important for the application of LAO as antitumor drugs.

The studies in the field of glycobiology in 2015 focused on lectins from different mushrooms and their effects on different cell lines, with the emphasis on immune cells as well as on their application as tools for targeted drug delivery. For the latter we prepared different fusion proteins consisting of lectins and cysteine protease inhibitors. We showed that lectin MpL, isolated from Macrolepiota procera, rapidly enters targeted lymphocytes by means of clatrin-dependent endocytosis. Inside the cell it triggers either lysosomes or Golgi apparatus. Fusion proteins consisted of MpL and cystatin C, a potent inhibitor of cysteine proteases, internalized targeted tumour cells and accumulated in lysosomes, where it significantly impaired the degradation of the extracellular matrix and consequently the invasiveness of tumour cells. On the other hand, a similar lectin CNL, isolated from Clitocybe nebularis, showed a different effect: by binding to membrane CD markers on lymphocytes it triggered the cell apoptosis. The results confirm the great potential of lectins for direct anti-tumour treatment or as a part of systems for the delivery of other anti-tumour drugs.

Figure 1: Amanita muscaria as a source of various potential anti-tumour compounds

Investigating the role of proteolytic enzymes in the regulation of the cytotoxic activity of immune cells we published in Oncotarget the results on the direct link between the activities of cystatin F, cathepsin C and granzyme B and the cytotoxicity of natural killer cells (NK cells). By using different cystatin F mutants we showed that the monomeric form of cystatin F, which is truncated at N-terminal end and fully glycosylated, is a key regulator of cytotoxicity. Additionally, we evaluated the contribution of other cells, in particular immune ones, which secrete cystatin F, on the function of NK cells and on their reduced cytotoxicity. A similar mechanism was observed for cytotoxic T lymphocytes, which after the contact with tumour cells also lose their cytotoxicity. We implemented a new method for an assessment of cytotoxicity by using calcein and proximity ligation assay for the assessment of the co-localisation and interaction between proteins. Using the latter we confirmed the interactions between cystatin F, cathepsin C and granzyme B in cytotoxic T lymphocytes.

In the field of molecular neurobiology, we published five research studies in reputable journals on molecular processes underlying frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS). We presented the atypical DNA secondary structures formed by the hexanucleotide repeat GGGGCC, which is found in the C9orf72 gene. The expansion mutation of this hexanucleotide repeat is the most common genetic cause of ALS and FTD (Neurobiology of Ageing) and the

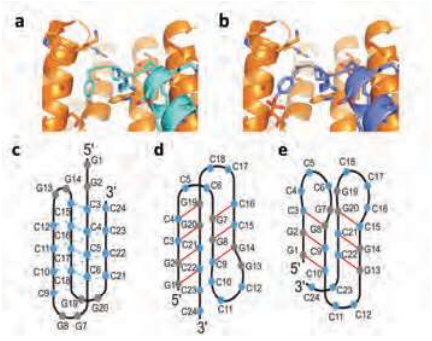

Figure 2: Structural model of the binding of unphosphorylated (a) and phosphorylated (b) C-terminal tyrosine of FUS to the transporting portein TNPO1 and i-motif (c) and two possible protonated hairpins (d and e) formed form the CCCGGG repeat DNA.

- **Cystatin F regulates the function of natural killer (NK) cells**
- **Lectins from mushrooms can be used in anti-tumour therapy**

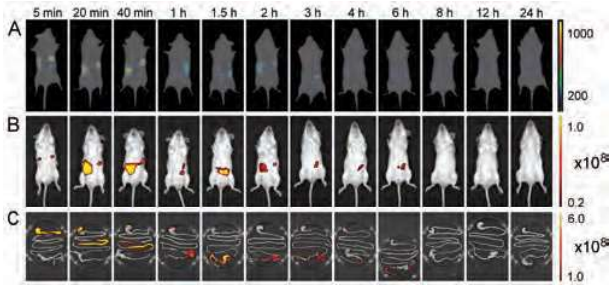

Figure 3: Imaging of mice at different time points after administration of 5.0×1010 cells of IRFP713-expressing L. lactis using trans-illumination and fluorescence imaging tomography (a) or epifluorescence (b) and ex vivo epifluorescence imaging of isolated intestines (c). Colour bars indicate radiant efficiency or total fluorescent yield.

- **Mutation in hexanucleotide repeat GGGGCC in the C9orf72 gene, which results in repeat expansion, is the most common genetic cause of frontotemporal dementia and amyotrophic lateral sclerosis**
- **Fluorescent tomography was confirmed as a suitable method for the spatial localization of bacteria in mice**
- **A review paper was published in Trends in Biotechnology**

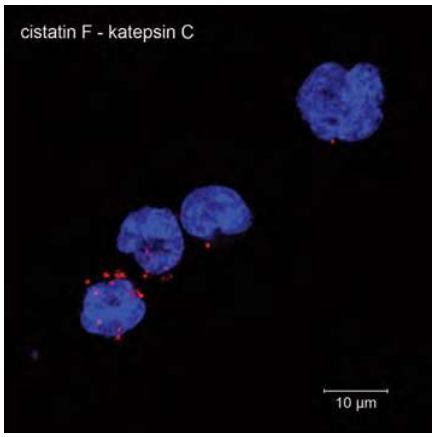

Figure 4: Co-localisation of cathepsin C and cystatin F in TALL-104 cells obtained by proximity ligation method.

reverse complement sequence, CCCCGG forms protonated hairpins and i-motifs (Scientific Reports). Of special interest was our observation that the equimolar mix of sense and anti-sense hexanucleotide repeat sequences preferentially forms single stranded G-quadruplex or i-motif structures instead of the expected DNA double helix. Additionally, we have shown that the C-terminal tyrosine of FUS can be phosphorylated, which may have implications for FUS-related FTD (Journal of Cell Science). We have also published the effect of the loss of TDP-43 on the proteome (Neuroscience) and screening of the Slovenian ALS patient population for the most common mutations (Neurobiology of Ageing).

In the field of the research of lactic acid bacteria we expressed infrared fluorescent protein IRFP713 in the bacteria Lactococcus lactis, Lactobacillus plantarum and Escherichia coli. IRFP713 has absorption and excitation maxima in the infrared part of the spectrum. The characteristic of infrared light is better penetration into the tissue in comparison to visible light. This has enabled us to monitor the bacteria in living mice with the use of fluorescence. We have determined that there are no major differences in intestinal transit times between different species of bacteria. Bacteria passed through the stomach and small intestine during the first hour after administration and were retained in the caecum and the large intestine for 6–8 h. The spatial localization of bacteria was confirmed by imaging of the isolated intestine and by cultivating of the intestinal content. We have also confirmed the suitability of fluorescent tomography for the spatial localization of bacteria in mice. Additionally, we have expressed another fluorescent protein, IRFP682, which has enabled concomitant in-vivo imaging of two different species of bacteria. With this work we laid the foundation for in-vivo imaging of lactic acid and commensal bacteria, as well as confirmed the suitability of the developed method for the acquisition of the information on temporal and spatial distribution of bacteria in the intestinal tract. This represents a basis for future therapeutic studies of probiotics.

We have also published a review article entitled "Non-immunoglobulin scaffolds: a focus on their targets" in which we reviewed 20 different types of binding proteins that can be randomized and that were not derived from the molecule of immunoglobulin. In the article we focused on an overview of more than 100 proteins that have served as targets for the selection of binding proteins. The review article was published in one of the top journals in the field of biotechnology, Trends in Biotechnology, with an impact factor of 11.9.

The results of the research work at the Department of Biotechnology in the year 2015 were published in 30 scientific papers in journals with an impact factor. Two patent applications were filed and a national patent was granted. We also received two research grants from the Slovenian Research Agency. A member of the department prof. Borut Štrukelj received a Zois Award for outstanding scientific achievements. Ph.D. student Simon Žurga received FEBS fellowship and Ph.D. students Ana Bajc Česnik and Simona Darovic Ad Futura fellowships, all for visiting foreign laboratories. Simon Žurga also received the Dean's Award for scientific achievement at the Faculty of Pharmacy, University of Ljubljana. The results of the study on cellular signalling of cathepsin X in cancer (Kos, J., Vižin, T., Pečar-Fonović, U., Pišlar, A., Seminars in Cancer Biology) have been selected and presented by the Slovenian Research Agency as Excellent in Science for the year 2015. Head of department prof. Janko Kos was elected as a member of European Academy of Sciences and Arts. The members of the department co-organized several scientific meetings (CITIM 2015, FEBS3+) and were also very active in pedagogical work as lecturers and mentors to students preparing diploma and doctoral theses at universities in Slovenia and abroad.

Some outstanding publications in the past year

1. Škrlec, Katja, Štrukelj, Borut, Berlec, Aleš. Non-immunoglobulin scaffolds: a focus on their targets. Trends in biotechnology, ISSN 0167-7799. [Print ed.], 2015, vol. 33, iss. 7, str. 408-418, IF 11,9
2. Pišlar, Anja, Perišić, Milica, Kos, Janko. Lysosomal cysteine peptidases - molecules signaling tumor cell death and survival. Seminars in cancer biology, ISSN 1044-579X, 2015, vol. 35, str. 168-179, IF 9,3

3. Magister, Špela, Tseng, Han-Ching, Bui, Vickie T., Kos, Janko, Jewett, Anahid. Regulation of split anergy in natural killer cells by inhibition of cathepsins C and H and cystatin F. Oncotarget, ISSN 1949-2553, Sep. 2015, vol. 6, no. 26, str. 22310-22327, IF 6,4

Awards and appointments

1. Borut Štrukelj: Zois Award 2015 for outstanding achievements in modern sustainable development of pharmaceutical biotechnology in the Republic of Slovenia, Portorož, Government of the Republic of Slovenia
2. Simon Žurga: Dean's Award 2015, for the paper "Biochemical properties of lectin from parasol mushroom (Macrolepiota procera) and its effects on model nematode Caenorhabditis elegans", published in the Federation of European Biochemical Societies (FEBS) Journal, Ljubljana, Faculty of Pharmacy, University of Ljubljana

Organization of conferences and meetings

1. Organization of the annual meeting of co-workers of the research programme "Pharmaceutical Biotechnology: Knowledge for Health" from the Department of Biotechnology, Jožef Stefan Institute, and the Chair of Pharmaceutical Biology, Faculty of Pharmacy, University of Ljubljana, Slovenia, 19 November 2015

Patent granted

1. Jana Erjavec, Tanja Dreo, Jerica Sabotič, Jože Brzin, Janko Kos, Maja Ravnikar, Composition and method for plant protection, SI24489 (A), Slovenian Intellectual Property Office, 30. 04. 2015.

INTERNATIONAL PROJECTS

1. Disrupted RNA Processing in Amyotrophic Lateral Sclerosis
   Prof. Boris Rogelj
   Slovenian Research Agency
2. Mechanism of C9orf72 extended Repeat Pathogenicity in ALS and FTD
   Prof. Boris Rogelj
   Slovenian Research Agency

RESEARCH PROGRAM

1. Pharmaceutical Biotechnology: Knowledge for Health
   Prof. Janko Kos

R & D GRANTS AND CONTRACTS

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

VISITORS FROM ABROAD

1. Prof. Pavle Andjus, Milena Milošević, Prof. Dušanka Savić Pavićević, Jovan Petrović, University of Belgrade, Faculty of Biology, Serbia, 14–17 May 2015.
2. Dr. Milena Milošević, University of Belgrade, Faculty of Biology, Serbia, 8–15 December 2015.

STAFF

Researchers
1. Prof. Janko Kos*, Head
2. Prof. Boris Rogelj
3. Dr. Jerica Sabotič
4. Prof. Borut Štrukelj*

Postdoctoral associates
5. Asst. Prof. Aleš Berlec
6. *Dr. Maruška Budič, left 01.03.15*
7. Dr. Anja Kovanda
8. Dr. Milica Perišić Nanut
9. Dr. Sonja Prpar Mihevc
10. Dr. Anja Pucer Janež
11. *Dr. Simon Žurga, left 01.10.15*

Postgraduates
12. Ana Bajc Česnik, B. Sc.
13. Simona Darovic, B. Sc.
14. Mateja Prunk, B. Sc.
15. Katja Škrlec, B. Sc.

Technical and administrative staff
16. Darja Žunič Kotar

BIBLIOGRAPHY

Original Article

1. Aleš Berlec, Janja Završnik, Miha Butinar, Boris Turk, Borut Štrukelj, "In vivo imaging of Lactococcus lactis, Lactobacillus plantarum and Escherichiacoli expressing infrared fluorescent protein in mice", *Microb Cell Fact*, vol. 14, no. 181, pp. 1-14, 2015.
2. Simona Darovic, Sonja Prpar Mihevc, Vera Župunski, Gregor Gunčar, Maja Štalekar, Youn-Bok Lee, Christopher E. Shaw, Boris Rogelj, "Phosphorylation of C-terminal tyrosine residue 526 in FUS impairs its nuclear import", *J Cell Sci*, vol. 128, no. 22, pp. 4151-4159, 2015.
3. Gorazd Drevenšek, Mojca Lunder, Eva Tavčar Benković, Ana Mikelj, Borut Štrukelj, Samo Kreft, "Silver fir (Abies alba) trunk extract protects guinea pig arteries from impaired functional responses and morphology due to an atherogenic diet", *Phytomedicine (Stuttg.)*, vol. 22, issue 9, pp. 856-861, 2015.
4. Natalia Guzior, Marek Bajda, Mirosław Skrok, Katarzyna Kurpiewska, Krzysztof Lewiński, Boris Brus, Anja Pišlar, Janko Kos, Stanislav Gobec, Barbara Malawska, "Development of multifunctional, heterodimeric isoindoline-1,3-dione derivatives as cholinesterase and ß-amyloid aggregation inhibitors with neuroprotective properties", *Eur. j. med. chem.*, vol. 92, pp. 738-749, 2015.
5. Anja Kovanda, Matjaž Zalar, Primož Šket, Janez Plavec, Boris Rogelj, "Anti-sense DNA d(GGCCCC)$_n$ expansions in C9ORF72 form i-motifs and protonated hairpins", *Scientific reports*, vol. 5, pp. 17944 -1-17944 -7, 2015.
6. Špela Magister, Han-Ching Tseng, Vickie T. Bui, Janko Kos, Anahid Jewett, "Regulation of split anergy in natural killer cells by inhibition of cathepsins C and H and cystatin F", *Oncotarget*, vol. 6, no. 26, pp. 22310-22327, Sep. 2015.
7. Bojana Mirković, Boštjan Markelc, Miha Butinar, Ana Mitrović, Izidor Sosič, Stanislav Gobec, Olga Vasiljeva, Boris Turk, Maja Čemažar, Gregor Serša, Janko Kos, "Nitroxoline impairs tumor progression in vitro and in vivo by regulating cathepsin B activity", *Oncotarget*, vol. 6, no. 22, pp. 19027-19042, 2015.
8. Jernej Oberčkal, Lidija Kovačič, Jernej Šribar, Adrijana Leonardi, Klemen Dolinar, Anja Pucer Janež, Igor Križaj, "On the role of protein disulfide isomerase in the retrograde cell transport of secreted phospholipases A_2", *PloS one*, vol. 10, no. 3, pp. e0120692-1-e0120692-20, 2015.
9. Jernej Ogorevc, Sonja Prpar Mihevc, Jakob Hedegaard, Dušan Benčina, Peter Dovč, "Transcriptomic response of goat mammary epithelial cells to Mycoplasma agalactiae challenge - a preliminary study", *Anim. Sci. Pap. Rep.*, vol. 33, no. 2, pp. 155-163, 2015.
10. Mitja Ogrizek, Samo Turk, Samo Lešnik, Izidor Sosič, Milan Hodošček, Bojana Mirković, Janko Kos, Dušanka Janežič, Stanislav Gobec, Janez Konc, "Molecular dynamics to enhance structure-based virtual screening on cathepsin B", *J. comput.-aided mol. des.*, vol. 29, iss. 8, pp. 707-712, 2015.
11. Urša Pečar Fonović, Janko Kos, "Cathepsin X cleaves profilin 1 C-Terminal Tyr139 and influences clathrin-mediated endocytosis", *PloS one*, vol. 10, no. 9, pp. 1-14, 2015.
12. Matjaž Ravnikar, Matic Terčelj, Damjan Janeš, Borut Štrukelj, Samo Kreft, "Antibacterial activity of endophytic fungi isolated from conifer needles", *Afr. j. biotechnol.*, vol. 14, no. 10, pp. 867-871, 2015.
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14. Primož Šket, Jure Pohleven, Anja Kovanda, Maja Štalekar, Vera Župunski, Matja Zalar, Janez Plavec, Boris Rogelj, "Characterization of DNA G-quadruplex species forming from C9ORF72 G_4C_2 expanded repeats associated with amyotrophic lateral sclerosis and frontotemporal lobar degeneration", *Neurobiol. aging*, vol. 36, no. 2, pp. 1091-1096, 2015.
15. Ida Šmid, Ana Rotter, Kristina Gruden, Jože Brzin, Meti Buh Gašparič, Janko Kos, Jana Žel, Jerica Sabotič, "Clitocypin, a fungal cysteine protease inhibitor, exerts its insecticidal effect on Colorado potato beetle larvae by inhibiting their digestive cysteine proteases", *Pestic. biochem. physiol.*, vol. 122, pp. 59-66, 2015.
16. Maja Štalekar, Xiaoke Yin, Katja Rebolj, Simona Darovic, Claire Troakes, Manuel Mayr, Christopher E. Shaw, Boris Rogelj, "Proteomic analyses reveal that loss of TDP-43 affects 3 RNA processing and intracellular transport", *Neuroscience*, vol. 293, pp. 157-170, 2015.
17. Katarina Vrabec, Blaž Koritnik, Lea Leonardis, Leja Dolenc-Grošelj, Janez Zidar, Bradley Smith, Caroline Vance, Boris Rogelj, Damjan Glavač, Metka Ravnik-Glavač, "Genetic analysis of amyotrophic lateral sclerosis in the Slovenian population", *Neurobiol. aging*, vol. 36, iss. 3, pp. 1601.e17-1601.e20, Mar. 2015.
18. Petra Zadravec, Borut Štrukelj, Aleš Berlec, "Heterologous surface display on lactic acid bacteria: non-GMO alternative?", *Bioengineered*, vol. 6, issue 3, pp. 179-183, 2015.
19. Petra Zadravec, Borut Štrukelj, Aleš Berlec, "Improvement of LysM-mediated surface display of DARPins in recombinant and non-recombinant Lactococcus lactis and Lactobacillus species", *Appl. environ. microbiol.*, vol. 81, no. 6, pp. 2098-2106, 2015.
20. Simon Žurga, Jure Pohleven, Janko Kos, Jerica Sabotič, "β-trefoil structure enables interactions between lectins and protease inhibitors that regulate their biological function", *J. Biochem. (Tokyo)*, vol. 158, issue 1, pp. 83-90, 2015.

Review Article

1. Janko Kos, Tjaša Vižin, Urša Pečar Fonović, Anja Pišlar, "Intracellular signaling by cathepsin X: molecular mechanisms and diagnostic and therapeutic opportunities in cancer", *Semin. cancer biol.*, vol. 31, pp. 76-83, 2015, .
2. Anja Pišlar, Milica Perišić, Janko Kos, "Lysosomal cysteine peptidases - molecules signaling tumor cell death and survival", *Semin. cancer biol.*, vol. 35, pp. 168-179, 2015.
3. Katja Škrlec, Borut Štrukelj, Aleš Berlec, "Non-immunoglobulin scaffolds: a focus on their targets", *Trends biotechnol. (Regul. ed.)*, vol. 33, iss. 7, pp. 408-418, 2015.
4. Borut Štrukelj, "Biološka zdravila pri multipli sklerozi", *Farm. vestn.*, vol. 66, no. 1, pp. 80-84, feb. 2015.
5. Borut Štrukelj, "Varnost, kakovost in učinkovitost originalnih in podobnih bioloških zdravil", *Farm. vestn.*, vol. 66, no. 3, pp. 256-259, avg. 2015.
6. Tjaša Vižin, Janko Kos, "Gamma-enolase: a well known tumour marker, with a less-known role in cancer", *Radiol. oncol.*, vol. 49, no. 3, pp. 217-226, Sep. 2015.
7. Miha Vodnik, Borut Štrukelj, Mojca Lunder, "Ghrelin receptor ligands reaching clinical trials: from peptides to peptidomimetics; from agonists to antagonists", *Horm. Metab. Res.*, vol., no., pp., 2015.

Professional Monograph

1. Jovan Miljković, Uroš Potočnik, Matjaž Deželak, Aleksandar Godić, Mateja Dolenc-Voljč, Iztok Holc, Liljana Mervic, Vesna Tlaker Žunter, Borut Štrukelj, Vita Dolžan, Žiga Rotar, *Monografija o luskavici*, Maribor, Združenje slovenskih dermatovenerologov, 2015.

Patent Application

1. Jana Erjavec, Tanja Dreo, Jerica Sabotič, Jože Brzin, Janko Kos, Maja Ravnikar, *Composition and method for plant protection*, WO2015058944 (A1), World Intellectual Property Organization, 30. 04. 2015.
2. Tadej Rejc, Uroš Petrič, Dora Debeljak, Toni Bremec, Polonca Ferk, Mojca Lunder, Irena Roškar, Borut Štrukelj, Samo Kreft, *Zmes naravnih polifenolov iz lesa bele jelke za zmanjšanje postprandialne glukoze*, P-201500116, Urad RS za intelektualno lastnino, 12. 5. 2015.
3. Miha Vodnik, Mojca Lunder, Borut Štrukelj, Eva Knuplež, Valentina Kubale, *Peptidi za farmakološko poseganje v grelinski sistem*, P-201500141, Urad RS za intelektualno lastnino, 8. 6. 2015.

Patent

1. Jana Erjavec, Tanja Dreo, Jerica Sabotič, Jože Brzin, Janko Kos, Maja Ravnikar, *Composition and method for plant protection*, SI24489 (A), Urad RS za intelektualno lastnino, 30. 04. 2015.

Mentoring

1. Urša Pečar Fonović, *Vloga katepsina X pri uravnavanju delovanja profilina 1 v tumorskih celičnih linijah:* doctoral dissertation, Ljubljana, 2015 (mentor Janko Kos).
2. Maja Štalekar, *Regulatory function of TDP-43 and implications in pathological changes in neurodegenerative diseases:* doctoral dissertation, Ljubljana, 2015 (mentor Boris Rogelj).

3. Tjaša Vižin, *The role of gamma-enolase in cancer and its regulation by proteolytic enzymes:* doctoral dissertation, Ljubljana, 2015 (mentor Janko Kos).
4. Simon Žurga, *Biochemical properties and function of ricin B like lectin from mushroom Macrolepiota procera:* doctoral dissertation, Ljubljana, 2015 (mentor Janko Kos; co-mentor Jerica Sabotič).
5. Sara Božič, *Comparison of bioluminiscent Escherichia coli K-12/pTetLux and WZM120/pCGLS-11 strains in the evaluation of antibacterial activity:* master's thesis, Ljubljana, 2015 (mentor Janko Kos; co-mentor Päivi Tammela).
6. Sara Redenšek, *Validation of a non-invasive screening test for diagnostics of Down syndrome from whole blood samples of pregnant women:* master's thesis, Ljubljana, 2015 (mentor Borut Štrukelj).
7. Gregor Vidovič, *Testing of plasmid DNA containing collagen promoter for gene delivery to selected tissue:* master's thesis, Ljubljana, 2015 (mentor Borut Štrukelj; co-mentor Gregor Serša).