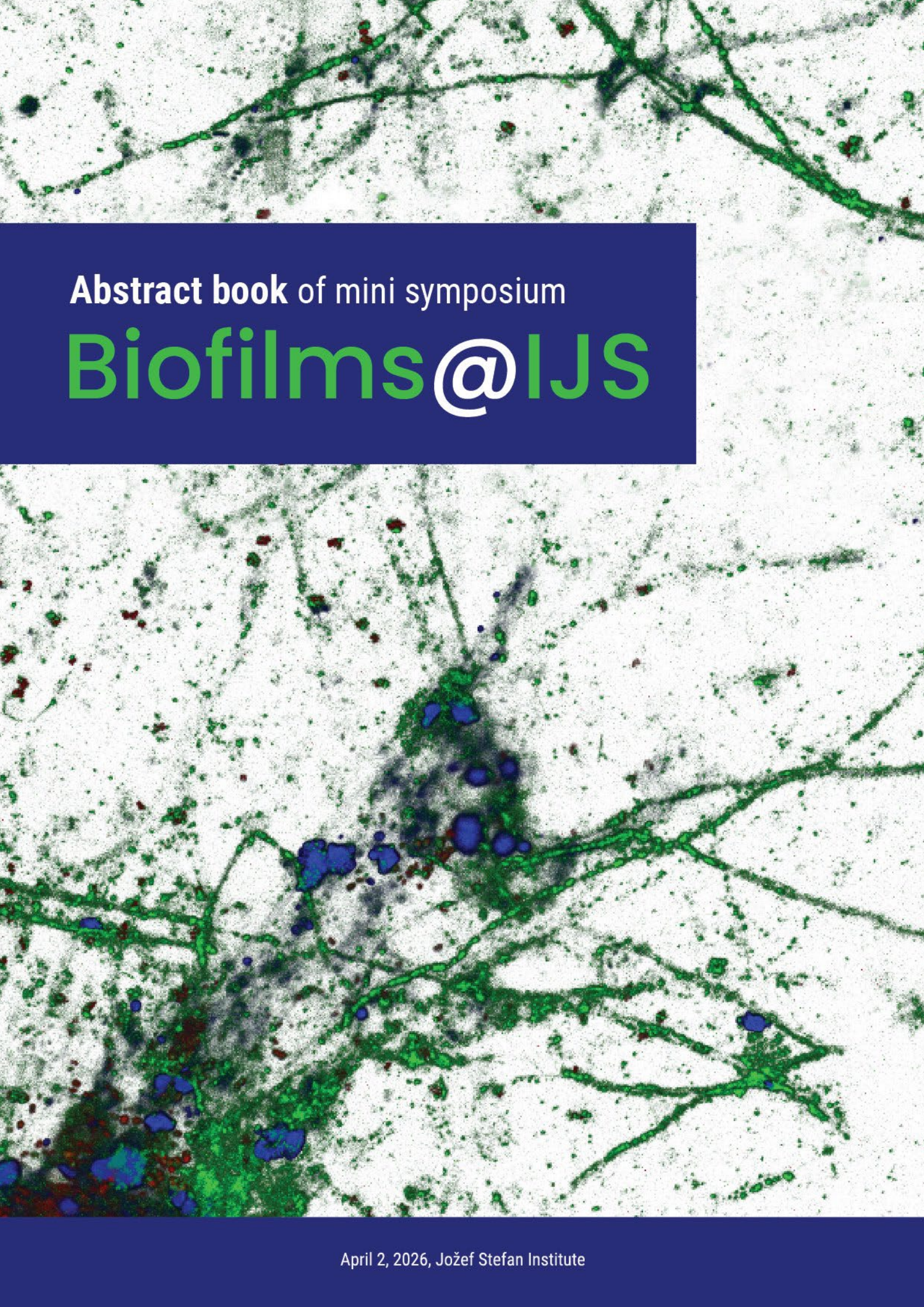


Abstract book of mini symposium

Biofilms@IJS

April 2, 2026, Jožef Stefan Institute

Abstract book of mini symposium

Biofilms@IJS

Organizer	Department of Biotechnology, Jožef Stefan Institute
Venue	Jožef Stefan Institute, Main Lecture Hall, Jamova 39, Ljubljana
Editor	Dr. Jerica Sabotič
Technical editor	Dr. Jerica Sabotič
Cover design	Lenka Trdina
Issued by	Department of Biotechnology, Jožef Stefan Institute, Ljubljana, Slovenia
Year of issue	2026
Online edition	Abstract book

Ljubljana, 2 April 2026

Kataložni zapis o publikaciji (CIP) pripravili v Narodni in univerzitetni knjižnici v Ljubljani

COBISS.SI-ID 273955075

ISBN 978-961-264-344-7 (PDF)

FOREWORD: Communities in Biofilm Research

Often invisible to the naked eye yet shaping ecosystems across the globe, biofilms are communities of microorganisms whose cooperative behavior has allowed them to survive, adapt, and thrive for billions of years in extreme and complex environments. Their resilience and versatility make them both a formidable challenge and an unparalleled opportunity for science and industry. Understanding these complex microbial communities requires researchers to adopt a similarly integrative approach, working across disciplines to explore, understand, and manage biofilms in natural and engineered systems.

Biofilms influence a wide range of processes, from nutrient cycling and biomineral formation in aquatic environments to applications in biotechnology and environmental sustainability. At the same time, they pose serious challenges, contributing to chronic infections, industrial contamination, and biofouling, where their intrinsic tolerance to antimicrobials complicates control strategies. Addressing this duality, and harnessing the benefits while mitigating risks, requires perspectives from microbiology, materials science, environmental science, biomedical engineering, and related fields.

This minisymposium exemplifies such interdisciplinarity, bringing together researchers with diverse expertise to examine the multifaceted nature of biofilms. The programme covers a wide range of topics, including biofilms in aquatic systems, genomic and phenotypic diversity of clinically and environmentally relevant bacteria, interspecies interactions within biofilm communities, and advances in synthetic and model biofilm systems. Presentations also highlight structural and mechanical properties of biofilms, nanomaterial-based control strategies, functionalized surfaces, magneto-mechanical disruption, novel antimicrobial compounds, and innovative experimental and analytical tools for investigating biofilm formation and eradication.

The event will feature a guest lecture by Prof. Stefano Fazi, PhD from the Water Research Institute (IRSA), National Research Council (CNR), Rome, Italy, who will provide insights into the dynamic roles of biofilms in aquatic environments.

The primary goal of this minisymposium is to connect research groups at the Jožef Stefan Institute and the University of Ljubljana, showcase ongoing work, and strengthen the interdisciplinary biofilm research community. Just as microorganisms in biofilms achieve resilience through cooperative behavior, researchers can address complex challenges and seize emerging opportunities by forming connected, cross-disciplinary networks. By facilitating dialogue, promoting idea exchange, and fostering new collaborations, this event marks an important step toward a more integrated understanding of biofilms and their roles in both natural and engineered systems.

dr. Jerica Sabotič
Organizer of the Biofilms@IJS Minisymposium

PROGRAMME

LECTURE ABSTRACTS

INVITED LECTURE: Dynamic roles of biofilms in aquatic environments: linking microbial communities to biomineral formation

Stefano Fazi
Water Research Institute (IRSA), National Research Council of Italy (CNR), Rome, Italy

Natural biofilms in aquatic ecosystems represent highly complex and dynamic microbial communities that play a fundamental role in ecosystem functioning. This presentation will explore approaches and methodologies for studying biofilms in natural environments, with a focus on their structural organization, ecological roles, and taxonomic and functional diversity as revealed by metagenomic analyses.
By integrating microbial ecology with biogeochemical perspectives, the talk will highlight how natural biofilms mediate key processes across scales. Particular attention will be given to biofilm-mediated carbonate bioprecipitation, illustrating how microbial activity drives mineral formation and contributes to nature-based carbon capture and sequestration.
These examples aim to provide a broader, system-level understanding of biofilms as essential components of aquatic ecosystems, bridging living microbial communities and mineral-forming processes.

Phenotypic and genomic characterization of clinical animal *Pseudomonas aeruginosa* isolates highlights biofilm formation and genetic diversity

Petra Seliškar[1], Bojan Papić[2], Darja Kušar[2], Martina Hrast Rambaher[1], Majda Golob[2]

[1] *University of Ljubljana, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Aškerčeva 7, Ljubljana, Slovenia*
[2] *University of Ljubljana, Veterinary Faculty, Institute of Microbiology and Parasitology, Gerbičeva ulica 60, Ljubljana, Slovenia*

Pseudomonas aeruginosa is an opportunistic pathogen capable of infecting a wide range of animal species, particularly when host defenses are compromised. This bacterium is ubiquitous in the environment, resistant to unfavorable conditions, and capable of spreading between humans and other mammals. In veterinary medicine, it is associated with infections such as otitis, wound and skin infections, urinary tract infections, mastitis and respiratory infections. *P. aeruginosa* is notable for its intrinsic resistance to many antimicrobials and its ability to acquire additional resistance mechanisms, which further complicates treatment. Its capacity for biofilm formation is a key virulence trait, enhancing persistence in host tissues and on surfaces and increasing tolerance to antimicrobials and host immune responses.

In this study, 100 *P. aeruginosa* isolates originating from various clinical samples and animal species (mainly pets, predominantly dogs and cats, but also a snake and a sea lion) underwent whole-genome sequencing and phenotypic screening for biofilm formation. The isolates exhibited high genetic diversity. Phenotypically, 53 % of isolates were strong biofilm formers, 12 % moderate, 13 % weak, and 22 % did not form biofilms.

These findings highlight the substantial genetic diversity of *P. aeruginosa* isolates from animals and emphasize the high prevalence of strong biofilm-forming phenotypes, which may contribute to persistence and complicate treatment in veterinary infections.

Extracting predictive structural features from *Listeria monocytogenes* biofilms for strain differentiation and trait prediction

Nika Janež[1]*, Blaž Škrlj[2]*, Aljaž Osojnik[2]*, Márta Ladányi[3], Martin Breskvar[2], Matej Petkovič[2], Boštjan Kokot[4,5], Petra Čotar[4], Bojan Papić[6], Majda Golob[6], Tjaša Peternel[1], Jerica Sabotič[1]

1 *Department of Biotechnology, Jožef Stefan Institute, Jamova cesta 39, Ljubljana, Slovenia*
2 *Department of Knowledge Technologies, Jožef Stefan Institute, Jamova cesta 39, Ljubljana, Slovenia*
3 *Department of Applied Statistics, Institute of Mathematics and Basic Science, Hungarian University of Agriculture and Life Sciences (MATE), Villányi street 29-43, Budapest, Hungary*
4 *Department of Condensed Matter Physics, Jožef Stefan Institute, Jamova cesta 39, Ljubljana, Slovenia*
5 *Centre for Advanced Optical Microscopies, Jožef Stefan Institute, Jamova cesta 39 Ljubljana, Slovenia*
6 *University of Ljubljana, Veterinary Faculty, Institute of Microbiology and Parasitology, Gerbičeva ulica 60, Ljubljana, Slovenia*
* authors contributed equally to the study

Machine learning excels at identifying patterns in complex systems such as biofilms, which are microbial communities with spatially defined three-dimensional (3D) structures. To capture spatial organization, non-imaging approaches provide only bulk information, so imaging-based methods, which reveal detailed distributions, are essential for studying bacterial structural organisation. Although imaging is feasible, automated analysis for reliable quantitative structural data remains limited and challenging.
We present MicroICS, an open-source pipeline that automates structural feature extraction from 3D biofilm images and applies machine learning for strain classification from images alone. Focusing on *Listeria monocytogenes* – a persistent, high-mortality foodborne pathogen – we acquired optical 0.5 µm thick sections across 10 µm of Syto9-stained biofilms from eight diverse *L. monocytogenes* strains, generating 3D images from which we extracted over 2,700 quantitative features. Machine learning models assessed the ability to distinguish strains based on these features, independently of technical and biological variability. An optimised random forest classifier achieved human-level accuracy on new input images, including images of perturbed structures from strains exposed to food extracts. Feature importance analysis using Gini impurity identified fractal dimension (biofilm complexity) and void fraction (density) as top predictors. We extended the framework to link biofilm structures to traits such as clinical associations, with feature importance rankings revealing key trends – such as the dominance of biofilm abundance over textural features – that highlight potential drivers of structural change and strain-specific responses.

Acknowledgements
This study was funded by the Slovenian Research and Innovation Agency [grants J4-4555, J4-60079, P4-0432 and infrastructure funding PR-12365] and received funding from the European Union's Horizon Europe research and innovation programme [grant agreement No. 101214398 (ELLIOT)].

Biofilm interactions between *Lactococcus cremoris* and *Campylobacter jejuni*

Blaž Jug[1], Maja Šikić Pogačar[2], Aleš Berlec[3,4], Anja Klančnik[1]

[1] *Biotechnical Faculty, Department of Food Science, University of Ljubljana, Jamnikarjeva 101, Ljubljana, Slovenia*
[2] *Faculty of Medicine, Department of Pediatrics, University of Maribor, Taborska Ulica 8, Maribor, Slovenia*
[3] *Jožef Stefan Institute, Department of Biotechnology, Jamova 39, Ljubljana, Slovenia*
[4] *Faculty of Pharmacy,University of Ljubljana, Aškerčeva 7, Ljubljana*

Campylobacter jejuni is the most common foodborne pathogen, and its persistence in food processing environments is closely linked to its ability to form biofilms. In our laboratory, we assess *C. jejuni* biofilm formation using a range of phenotypic and molecular approaches, including model systems that simulate food processing conditions, abiotic surfaces relevant to food production, and biotic surfaces associated with the gastrointestinal tract or with extracellular matrices produced by other bacteria, such as food spoilers and food fermenters. We have also optimised transcriptomic and proteomic workflows for the analysis of *C. jejuni* biofilm cells to better understand the genes and pathways associated with biofilm development, growth and resistance to different classical and alternative disruptive methods. In this study, we focused on the interaction between *C. jejuni* and the transient bacterium with probiotic properties *Lactococcus cremoris*. We investigated the effect of *L. cremoris* on *C. jejuni* biofilm formation on biotic and abiotic model surfaces, as well as on virulence, culturability, and proteomic adaptation in co-culture. Adhesion and invasion assays using human colon adenocarcinoma Caco-2 cells showed that *L. cremoris* reduced *C. jejuni* adhesion by 50% and invasion by 92%, particularly when *L. cremoris* is present before pathogen inoculation. Proteomic analysis further showed that co-cultivation with *L. cremoris* induced broad metabolic adaptation in *C. jejuni*, including changes in proteins involved in energy metabolism, nutrient acquisition, chemotaxis, and oxidative and acid stress response. This shows the adaptive possibilities *C. jejuni* has to enhance its survival in dual species biofilms, even though its culturability was strongly reduced. Together, these results show that *L. cremoris* acts antagonistically toward *C. jejuni* during early host cell interaction and during biofilm associated persistence, supporting its relevance as a protective competitor in food and host associated microbial environments. Proteomic analysis of *C. jejuni* revealed a versatile adaptive capacity that may contribute to its environmental persistence and help explain why it remains such a troublesome foodborne pathogen in food processing environments.

Acknowledgement: This research was funded by the Slovenian Research and Innovation Agency (grant numbers J4-3088, J7-4420, J4-4548, and P4-0116).

Synthetic biofilms and aggregates - the colloid biology approach

Tomaž Rijavec, Dmitrii Deev, Iaroslav Rybkin, Maja Zugan, Kristjan Gašpirc, Tayebeh Sharifi, Rahuraj Singh Chouhan, Aleš Lapanje

Group for Colloid Biology, Department for Environmental Sciences, Jožef Stefan Institute, Jamova cesta 39, 1000 Ljubljana

Colloid biology is an emerging field alongside colloid physics and chemistry, where the colloids we are studying, i.e. microbial cells, are viable entities that respond to their environment resulting in dynamic physicochemical properties over time. Understanding microbial genetics, physiology and community structures, we can treat microbial cells as particles that can be used as building blocks for the construction of synthetic consortia, either as suspended aggregates or biofilms attached to surfaces. By depositing polyelectrolytes onto the cells surface, we can modify its surface charge to facilitate electrostatic aggregation of cells in suspension or electrostatic deposition of cells onto the surface of a material. The aggregated structures can simulate natural systems, flocks and biofilms, where bacterial cells are immobilized, and can serve as models for studying natural transformation processes.
The approach is important for studying the fundamental questions of bacterial physiology and it has a wide applicative potential ranging from bioremediation, biotechnology, agronomy and medicine. In combination with other functional materials, like nanostructured materials, material for electrodes in electrochemicals systems and porous cell carrier materials, we can devise complex bio-based hybrid solutions that incorporate targeted metabolite conversion.

Mechanical structure of the biofilm as a decisive factor in antimicrobial tolerance

Mojca Blaznik, Marko Volk, Barbara Kraigher, Ines Mandic-Mulec, David Stopar, Iztok Dogša

Chair of Microbiology, Department of Microbiology, Biotechnical Faculty, University of Ljubljana, Večna pot 111, Ljubljana

Bacterial biofilms exhibit high tolerance to antimicrobial agents, yet the role of their spatial organization and mechanical properties in this phenomenon is not fully understood. In this lecture, The findings from the model system *Bacillus subtilis* show that the structural and mechanical integrity of the biofilm is a key determinant of antimicrobial tolerance.
Using a combination of genetic mutants, mechanical disruption of biofilms, and exposure to the antibiotic daptomycin, we demonstrate that tolerance is closely linked to the integrity and viscoelastic properties of the extracellular matrix, particularly the exopolysaccharide EpsA-O. Mechanical disruption of the biofilm structure markedly increases the susceptibility of bacterial communities to antibiotics.
These results indicate that antimicrobial tolerance in biofilms is not solely a consequence of physiological heterogeneity, but is also importantly shaped by the collective structural and mechanical properties of the biofilm and its matrix.

Polymeric surfaces decorated by nanostructured Magnesium-oxide for antibiofilm activity

Nemanja Aničić[1], Samo Jeverica,[2] Matjaž Spreizter[1], Marija Vukomanović[1]

[1] *Advanced materials Department, Jožef Stefan Institute, Ljubljana, Slovenia*
[2] *National Laboratory of Health, Environment and Food, Prvomajska 1, 2000 Maribor, Slovenia*

MgO kills bacteria mainly through direct surface contact, and that effect depends on its atomic-scale surface defects [1]. The study shows that MgO with fewer low-coordinated surface oxygen atoms is more resistant to hydrolysis and also less antibacterial. ROS are not an intrinsic property of MgO; they appear only during hydrolysis of the most defective particles or during MgO–bacteria interaction. Overall, the results suggest that MgO's antibacterial action is driven largely by an acid–base reaction between the MgO surface and the bacterial cell wall. Nanotextured MgO is promising for implant protection because it can both kill bacteria and support tissue regeneration, but it needs a polymer carrier that preserves its active surface, slows conversion to less active $Mg(OH)_2$, and controls Mg release [2]. This study embedded MgO microrods into PLGA, PLA, and PCL matrices and found that the matrix strongly affects composite performance through differences in hydrophilicity, polarity, and degradation. PLGA performed best: its balance of MgO–polymer interactions and degradation kinetics, together with the 1D nanotextured MgO shape, gave the strongest antibacterial activity against planktonic and biofilm-forming bacteria, including MRSA and clinical implant-infection strains, while maintaining controlled magnesium ion release and no detectable red-blood-cell damage.

Acknowledgement
This work was supported by the J3-4531 project and the research program P2-0091.

[1] https://doi.org/10.1002/smll.201800205
[2] https://doi.org/10.3390/polym13132183

Piezoelectricity-driven antibacterial activity of poly(L-lactide) for limiting biofilm formation

Lea Gazvoda[1], Milica Peršić Nanut[1, 2], Matjaž Spreizter[1], Marija Vukomanović[1]

[1] *Advanced materials Department, Jožef Stefan Institute, Ljubljana, Slovenia*
[2] *Department of Biotechnology, Jožef Stefan Institute, Ljubljana, Slovenia*

Biofilm formation on polymeric biomaterials remains a major challenge in biomedical applications, particularly for implanted devices where bacterial colonization can lead to persistent infections. Poly(L-lactide) (PLLA), a semi-crystalline biodegradable and biocompatible polymer widely used in medical devices and tissue engineering, has recently attracted attention not only for its structural properties but also for its ability to influence bacterial adhesion and biofilm development through its piezoelectric properties [1]. Recent studies have demonstrated that piezoelectric PLLA films exhibit intrinsic antimicrobial activity without the use of antibiotics or inorganic nanoparticles. In particular, PLLA surfaces with enhanced piezoelectric properties can affect bacterial viability through electrical stimulation that disrupts the bacterial membrane potential [1]. This effect has been demonstrated against *Staphylococcus epidermidis*, a Gram-positive bacteria commonly associated with biofilm formation on medical devices, especially on highly nanotextured PLLA surfaces exhibiting increased piezoelectric properties [2]. Piezoelectric stimulation generated by mechanically ultrasound activated PLLA nanotubes resulted in membrane damage of bacteria and reduced bacterial survival, indicating a promising strategy for preventing bacterial colonization and subsequent biofilm development. An antibacterial response was also observed for *Pseudomonas aeruginosa* (wild type), a highly efficient biofilm-forming pathogen frequently associated with burn wound infections (Fig. 1).
These findings highlight the potential of piezoelectric material, among them also biodegradable PLLA as an antibiotic-free antimicrobial platform capable of limiting biofilm formation on biomaterial surfaces while maintaining biocompatibility for biomedical applications.

Acknowledgement
This work was supported by the J3-4531 project and the research program P2-0091.

[1] https://doi.org/10.3390/ijms24087473
[2] https://doi.org/10.3390/polym13040613

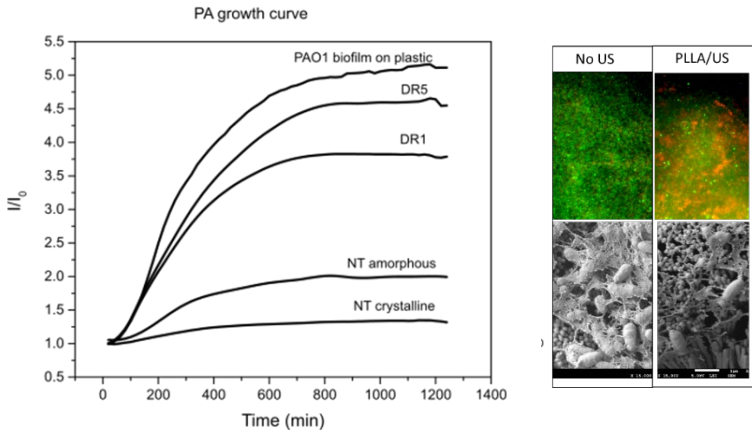


Figure 1. Relative growth curve of bacteria PAO1 in presence of piezoelectric or non-piezo materials with smooth or nanotextured structure (left) and live/dead and SEM images of bacteria grown on nanotextured sample surfaces for 2 days (right).

Functionalized gold nanoparticles as a promising platform against biofilm-forming bacteria

Martina Žabčić[1], Mitja Rak[2], Verica Mioč[3], Andrej Steyer[3], Matjaž Spreitzer[1], Marija Vukomanović[1]

[1] *Advanced Materials Department, Jožef Stefan Institute, Ljubljana, Slovenia*
[2] *Medical Microbiology Department, National Laboratory of Health, Environment and Food, Koper, Slovenia*
[3] *Public Health Microbiology Department, National Laboratory of Health, Environment and Food, Ljubljana, Slovenia*

Functionalized nanoparticles represent a promising strategy in combating bacterial infections, particularly those associated with increasing antibiotic resistance. In this study, surface-functionalized gold nanoparticles (AuNPs) on different templates, with contact-based mechanism, were synthesized in order to enhance their antimicrobial activity against both Gram-positive and Gram-negative bacteria, including bacteria species known for their biofilm-forming ability. The synthesized nanoparticles were characterized using various techniques to confirm their size, morphology, and surface functionalization.
Antimicrobial activity of AuNPs was evaluated using different methods, including following of the bacterial growth and determination of the minimum inhibitory concentration (MIC) and assessment of total growth reduction. The results demonstrated that the functionalized AuNPs exhibit strong antibacterial activity against a broad spectrum of bacteria, with effectiveness even against *Enterococcus*, both Vancomycin resistant and non-resistant strain, known to form biofilms. With TEM microscopy, interaction between *E. faecalis* biofilm and AuNPs was observed. Due to interactions between the nanoparticles and bacteria, cell membrane is disrupted which results in disruption of biofilm structure.
These findings indicate that functionalized gold nanoparticles have significant potential as a novel antimicrobial platform for controlling bacterial infections and preventing biofilm-associated pathogens.

Acknowledgements
This research was funded by Slovenian Research and Innovation Agency within grants J3-4531, PR-12591 and P2-0091.

Magneto-mechanical loosening of bacterial biofilms using anisotropic magnetic micro- and nanorobots

Maja Caf[1,2], Vincent Pautu[3], Charlotte Da Silva[4], Nika Zaveršek[5], Parvaneh Esmaeilnejad-Ahranjani[1], Sebastjan Nemec[1], Alenka Mertelj[1], Laurine Marger[6], Fabrice Marger[6], Mustapha Mekki[6], Irena Milošević[3], Jerica Sabotič[5], Stane Pajk[2], Aleš Berlec[1,2], Jelena Kolosnjaj-Tabi[4] and Slavko Kralj[1,2]*

1 *Department for Materials Synthesis, Jožef Stefan Institute, Jamova cesta 39, Ljubljana, Slovenia*
2 *University of Ljubljana, Faculty of Pharmacy, Aškerčeva cesta 7, Ljubljana, Slovenia*
3 *School of Engineering, Architecture and Landscape of Geneva (HEPIA), HES-SO - University of Applied Sciences and Arts Western Switzerland*
4 *Institut de Pharmacologie et de Biologie Structurale (IPBS), Université de Toulouse, CNRS, Université Toulouse III-Paul Sabatier (UPS), Toulouse 31400, France*
5 *Department of Biotechnology, Jožef Stefan Institute, Jamova cesta 39, Ljubljana, Slovenia*
6 *University Clinics of Dental Medicine, University of Geneva, Geneva, Switzerland*

Bacterial biofilms are structured microbial communities embedded within an extracellular polymeric substance (EPS) matrix that acts as a protective barrier, limiting antibiotic penetration and promoting antimicrobial resistance. Consequently, new strategies capable of mechanically disrupting biofilm architecture are of increasing interest [1]. In this work, we explore magneto-mechanical biofilm loosening using anisotropic magnetic particles actuated by low-frequency rotating magnetic fields (RMF).

First, we investigate the influence of particle size, shape, and magnetic properties on torque-driven biofilm removal. Magnetic microrods, nanochains, and nanorods with distinct dimensions were actuated under identical RMF conditions on implant-relevant titanium substrates colonized by *Enterococcus faecalis*. Micron-scale magnetic microrods generated sufficient magnetic torque to mechanically disrupt the EPS matrix and detach biofilm structures, significantly increasing the number of suspended bacterial cells without pronounced bactericidal effects. In contrast, nanoscale particles did not induce significant biofilm detachment but caused membrane changes/damage and increased the proportion of injured bacterial cells, indicating a size-dependent transition between macroscale biofilm detachment and nanoscale membrane interactions.

In another study, we introduced dynamically assembling magnetic nanochains as bioinspired swarm-type nanorobots capable of penetrating planktonic biofilms [2]. Under low-intensity RMF (<20 mT, <10 Hz), the nanochains exhibit propelling and rotational motion, enabling their attachment to bacterial aggregates and multidirectional penetration throughout *Staphylococcus epidermidis* biofilms. The resulting torque-driven mechanical interactions destabilize and loosen the EPS matrix and significantly enhance antibiotic penetration, leading to near-complete (99.99%) bacterial eradication when combined with methicillin.

These results demonstrate that anisotropic magnetic micro- and nanoscale structures represent promising magnetically actuated tools for targeted biofilm disruption and improved antimicrobial therapies.

Acknowledgement: This research was funded by the Slovenian Research and Innovation Agency (ARIS) through the core funding No. P2-0089, P4-0432 and ARIS projects: No. J2-60047, J1-70022, J2-3043, J3-3079, J7-4420, L2-60141.

[1] M. Caf et al., Magnetic field-driven strategies for biofilm disruption: From iron oxide nanoparticles to adaptive swarms of magnetic microrobots, ACS Nano 2026, 20, 1, 34–58
[2] S. Kralj et al., Dynamically Assembling Magnetic Nanochains as New Generation of Swarm-Type Magneto-Mechanical Nanorobots Affecting Biofilm Integrity Adv. Healthcare Mater. 2025, 14, 2403736

Development of biocidal magnetic nanorods for magnetic-field–induced biofilm eradication

Maja Caf[1,2], Nika Zaveršek[3], Stane Pajk[2], Jerica Sabotič[3], and Slavko Kralj[1,2]

[1] *Department for Materials Synthesis, Jožef Stefan Institute, Jamova cesta 39, Ljubljana, Slovenia*
[2] *University of Ljubljana, Faculty of Pharmacy, Aškerčeva cesta 7, Ljubljana, Slovenia*
[3] *Department of Biotechnology, Jožef Stefan Institute, Jamova cesta 39, Ljubljana, Slovenia*

Biofilms are microbial communities embedded in an extracellular polymeric substance (EPS) matrix that promotes surface adhesion and protects bacteria from antimicrobial agents, contributing to persistent infections and device-associated complications [1]–[3] . New strategies capable of disrupting biofilm structure and improving antimicrobial efficacy are therefore needed. Nanotechnology offers promising solutions, particularly through superparamagnetic iron oxide nanoparticles (SPIONs), which exhibit tunable surface chemistry, superparamagnetic behavior, and responsiveness to external magnetic fields. These properties enable biofilm disruption through different mechanisms as among them also mechanical perturbation of the EPS matrix and enhanced antimicrobial delivery [4].

In this study, we developed anisotropic magnetic nanoparticles (nanorods) designed for magnetically actuated biofilm removal by nanorods exposed to rotating magnetic field. Magnetic nanorods were assembled from superparamagnetic iron oxide nanocrystals under an external magnetic field, producing elongated nanostructures that were subsequently stabilized by a silica coating. The resulting nanorods exhibited average dimensions of 1.9 ± 0.7 μm in length and 94.3 ± 12.8 nm in width and retained superparamagnetic properties with a saturation magnetization of 18.5 emu g^{-1}, enabling efficient magneto-mechanical actuation.

The nanorods were further functionalized with three biocidal ligands possessing a permanent positive charge with variable hydrophobic characters. This design promotes electrostatic attraction to negatively charged bacterial surfaces and facilitates efficient association with the bacterial lipid bilayer, ultimately causing membrane disruption and cell rupture. The synthesized nanoparticles were characterized using transmission electron microscopy (TEM), vibrating sample magnetometry (VSM), and zeta potential measurements.

Acknowledgement: This research was funded by the Slovenian Research and Innovation Agency (ARIS) through the core funding No. P2-0089, P4-0432 and ARIS projects: No. J2-60047, J1-70022, J2-3043, J3-3079, J7-4420, L2-60141.

[1] H.-C. Flemming, J. Wingender, U. Szewzyk, P. Steinberg, S. A. Rice, and S. Kjelleberg, "Biofilms: an emergent form of bacterial life," *Nat. Rev. Microbiol.*, vol. 14, no. 9, pp. 563–575, Aug. 2016.
[2] K. Razdan, J. Garcia-Lara, V. R. Sinha, and K. K. Singh, "Pharmaceutical strategies for the treatment of bacterial biofilms in chronic wounds," *Drug Discov. Today*, vol. 27, no. 8, pp. 2137–2150, Aug. 2022.
[3] R. A. G. da Silva, I. Afonina, and K. A. Kline, "Eradicating biofilm infections: an update on current and prospective approaches," *Curr. Opin. Microbiol.*, vol. 63, pp. 117–125, Oct. 2021.
[4] M. Caf *et al.*, "Magnetic field-driven strategies for biofilm disruption: From iron oxide nanoparticles to adaptive swarms of magnetic microrobots," *ACS Nano*, no. acsna5c14390, Jan. 2026.

Hybrid nanomaterial methods for the disruption and removal of persistent *Listeria innocua* biofilms

Nika Zaveršek[1,2], Maja Caf[3,4], Vincent Pautu[5], Nika Janež[1], Irena Milosevic[5], Aleš Berlec[1,4], Slavko Kralj[3,4], Jerica Sabotič[1]

[1] *Jožef Stefan Institute, Department of Biotechnology, Ljubljana, Slovenia*
[2] *University of Ljubljana, Biotechnical faculty, Ljubljana, Slovenia*
[3] *Jožef Stefan Institute, Department for materials synthesis, Ljubljana, Slovenia*
[4] *University of Ljubljana, Faculty of Pharmacy, Ljubljana, Slovenia*
[5] *School of Engineering, Architecture and Landscape of Geneva (HEPIA), HES-SO - University of Applied Sciences and Arts, Western Switzerland*

Bacteria within biofilms are protected from environmental stressors and therefore exhibit high tolerance to conventional biofilm removal strategies, such as chemical disinfectants. The aim of this study was to systematically evaluate innovative approaches for the removal of persistent *Listeria innocua* biofilms. In particular, we investigated nanomaterial-based strategies combined with antibacterial or bacterial-surface-binding compounds and proteins that enable specific or non-specific binding to bacterial cells to enhance biofilm removal.

In our study we used anisotropic magnetic nanoparticles (AMPs) composed of iron oxide, that enable magneto-mechanical disruption of biofilms when exposed to a rotating magnetic field at relatively low rotational speeds. In addition to mechanical disruption, chemical and biochemical approaches were investigated using antibacterial silver nanoparticles, chemical and biochemical molecules with the ability to bind to the surface of bacterial cells. Efficacy was evaluated with classical microbiological methods such as colony-forming units and growth curve measurements.

The primary focus of this study was to evaluate how structural properties of AMPs, such as surface roughness and particle size, influence biofilm removal efficiency. A secondary objective was to determine whether combining magneto-mechanical disruption with chemical or biochemical strategies could produce synergistic effects. Our results highlight the importance of combining novel approaches for effective biofilm control. While AMP surface roughness did not significantly influence biofilm removal, particle size had a pronounced effect. Moreover, silver nanoparticles grafted onto the most effective AMPs generated a synergistic mechanical–chemical effect, significantly enhancing the disruption of *L. innocua* biofilms. This was also shown with bacterial-surface-binding compounds.

Overall, our findings demonstrate that magnetically activated hybrid nanomaterials, combined with antibacterial or bacterial-surface-binding compounds and proteins, represent a promising strategy for robust and reliable biofilm removal from surfaces.

Monobactam conjugates with antibiofilm activity

Vid Kavaš, Martina Hrast Rambaher

University of Ljubljana, Faculty of Pharmacy, Department of Pharmaceutical Chemistry

Antimicrobial resistance represents a major global health challenge, with drug-resistant bacterial infections associated with approximately 4.7 million deaths yearly. β-Lactam antibiotics remain the most widely used antibacterial agents; however, their efficacy is increasingly compromised by resistance mechanisms such as mutations in penicillin-binding proteins and the production of β-lactamases. Monobactams, a subclass of β-lactams characterized by a monocyclic core, are resistant to hydrolysis by metallo-β-lactamases, with aztreonam currently being the only clinically approved representative. [1] Targeting bacterial biofilms represents a promising strategy to combat antimicrobial resistance, as biofilms contribute to infection persistence and increased tolerance to antibiotic treatment. [2]
We synthesized two series of aztreonam chimeras targeting biofilm-related mechanisms. The first series comprised nitroxide conjugates (10 compounds) that mimic nitric oxide, while the second included acylhomoserine lactone (AHL) and acylhomocysteine lactone derivatives (14 compounds) designed to interfere with quorum sensing. The conjugates were prepared using various linkers forming either amide bonds (AHLs and nitroxides) or, in the case of AHL derivatives, additional sulfonic acid esters.
Some compounds exhibited MIC values comparable to aztreonam against selected ESKAPE pathogens, although their overall antibacterial activity was generally somewhat lower. The added value of these conjugates lies in their ability to inhibit biofilm formation in clinical isolates of *Escherichia coli*, *Pseudomonas aeruginosa* PAO1, and *Acinetobacter baumannii*. While their activity against mature biofilms was less pronounced, the results indicate their potential to affect both biofilm formation and, to a lesser extent, biofilm disintegration.

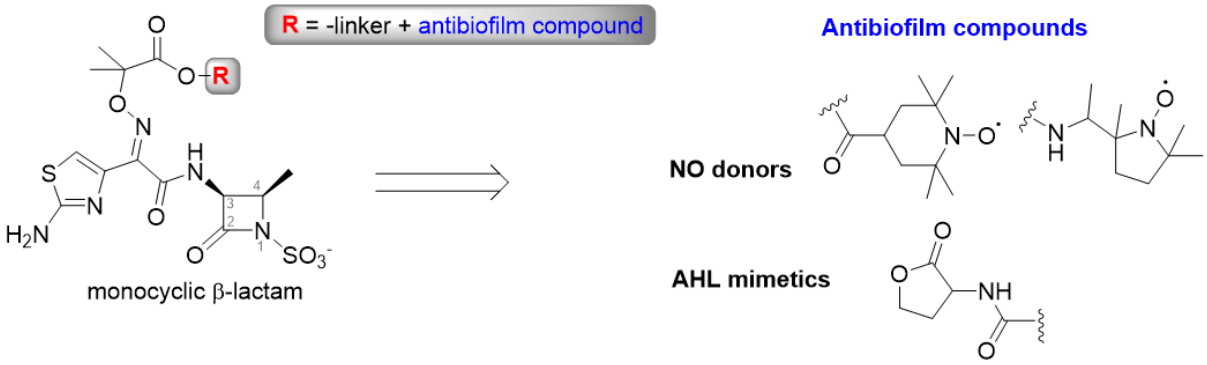


Figure 1: Monobactam-antibiofilm compound conjugates

[1] Lima LM et al., Eur J Med Chem, 2020, 208:112829
[2] Sharma D et al., Antimicrobial Resistance & Infection Control, 2019, 8(1):76

New tools for modulation of biofilm formation

Nika Janež[1], Meta Sterniša[2], Nika Zaveršek[1], Tjaša Peternel[1], Blaž Jug[2], Anja Klančnik[2], Jerica Sabotič[1]

[1] *Jožef Stefan Institute, Department of Biotechnology, Jamova cesta 39, Ljubljana, Slovenia*
[2] *Biotechnical Faculty, Department of Food Science, University of Ljubljana, Jamnikarjeva 101, Ljubljana, Slovenia*

Microbial glycobiology offers valuable opportunities to understand and control bacterial biofilms by targeting surface glycans, proteins, and extracellular matrix components. In our work, we focus on two important foodborne pathogens, *Listeria monocytogenes* and *Salmonella* Infantis, both of which are capable of forming persistent biofilms in food-production environments and contribute to contamination and food safety risks. Our research addresses this challenge from two complementary perspectives: using fungal lectins as molecular tools to investigate biofilm biology, and exploring higher fungi as sources of novel antibiofilm compounds.

Our primary model organism is *Listeria monocytogenes*, with *Listeria innocua* included as a surrogate microorganism for selected studies. Unlike many other bacteria, *Listeria* biofilms contain relatively low levels of extracellular polymeric substances (EPS), making cell-surface interactions especially important for adhesion and biofilm development. We have shown that fungal lectins and protease inhibitors can significantly impair *Listeria* biofilm formation without affecting bacterial growth, indicating a specific antibiofilm rather than antibacterial mode of action. Their effects are particularly pronounced during the initial stages of adhesion, suggesting that fungal proteins modulate interactions between bacterial cells and surfaces through recognition of surface glycans and proteins. Several fungal proteins also reduce biofilm formation on biologically relevant surfaces, highlighting their potential for future applications aimed at reducing contamination in food processing settings [1, 2, 3].

To complement these findings in *Listeria*, we also investigated *Salmonella* Infantis, another major foodborne pathogen with strong biofilm-forming capacity. Growth curve analysis was developed and employed to efficiently distinguish antimicrobial from antibiofilm activities across 42 fungal extracts, identifying *Pseudohydnum gelatinosum* as a source of antimicrobial activity and *Pleurotus ostreatus* as a source of specific antibiofilm compounds. Fractionation studies linked antimicrobial effects to protein-rich fractions containing L-amino acid oxidase activity, while the antibiofilm components remain to be identified [4, 5, 6].

Together, our studies demonstrate how fungal lectins and other fungal-derived molecules can serve both as tools for probing bacterial surface biology and as promising leads for the development of new strategies to control biofilms.

Acknowledgement: This research was funded by the Slovenian Research and Innovation Agency (ARIS) through the core funding P4-0432, P4-0116, and ARIS projects J4-1771, J4-9299, J4-4555, J4-60079, J4-70173.

[1] Janež N et al. Microb Biotechnol. 2024; 17(12):e70040. doi: 10.1111/1751-7915.70040.
[2] Bellich B & Janež N et al. Carbohydr Res. 2022; 511:108499. doi: 10.1016/j.carres.2021.108499.
[3] Janež N. Microb Biotechnol. 2021; 14(4):1269-1281. doi: 10.1111/1751-7915.13847.
[4] Sterniša M et al. Int J Food Microbiol. 2022; 364:109520. doi: 10.1016/j.ijfoodmicro.2021.109520.
[5] Sterniša M et al. Bio Protoc. 2023; 13(15): e4783. doi: 10.21769/BioProtoc.4783.
[6] Sterniša M et al. 2024; Patent family PCT/EP2022/058548 - WO2022/207781 - EP2024/4314318B1.

List of speakers

Maja Caf
Department for Materials Synthesis
Jožef Stefan Institute
Jamova cesta 39
Ljubljana

Iztok Dogša
Chair of Microbiology
Department of Microbiology
Biotechnical Faculty
University of Ljubljana
Večna pot 111
Ljubljana

Stefano Fazi
Water Research Institute (IRSA)
National Research Council of Italy (CNR)
Via Salaria km 29.300 - CP10
00015 Monterotondo
Rome
Italy

Lea Gazvoda
Advanced Materials Department
Jožef Stefan Institute
Jamova cesta 39
Ljubljana

Nika Janež
Department of Biotechnology
Jožef Stefan Institute
Jamova cesta 39
Ljubljana

Blaž Jug
Department of Food Science and Technology
Biotechnical Faculty
University of Ljubljana
Jamnikarjeva ulica 101
Ljubljana

Slavko Kralj
Department for Materials Synthesis
Jožef Stefan Institute
Jamova cesta 39
Ljubljana

Martina Hrast Rambaher
Department of Pharmaceutical Chemistry
Faculty of Pharmacy
University of Ljubljana
Aškerčeva ulica 7
Ljubljana

Tomaž Rijavec
Group for Colloid Biology
Department for Environmental Sciences
Jožef Stefan Institute
Jamova cesta 39
Ljubljana

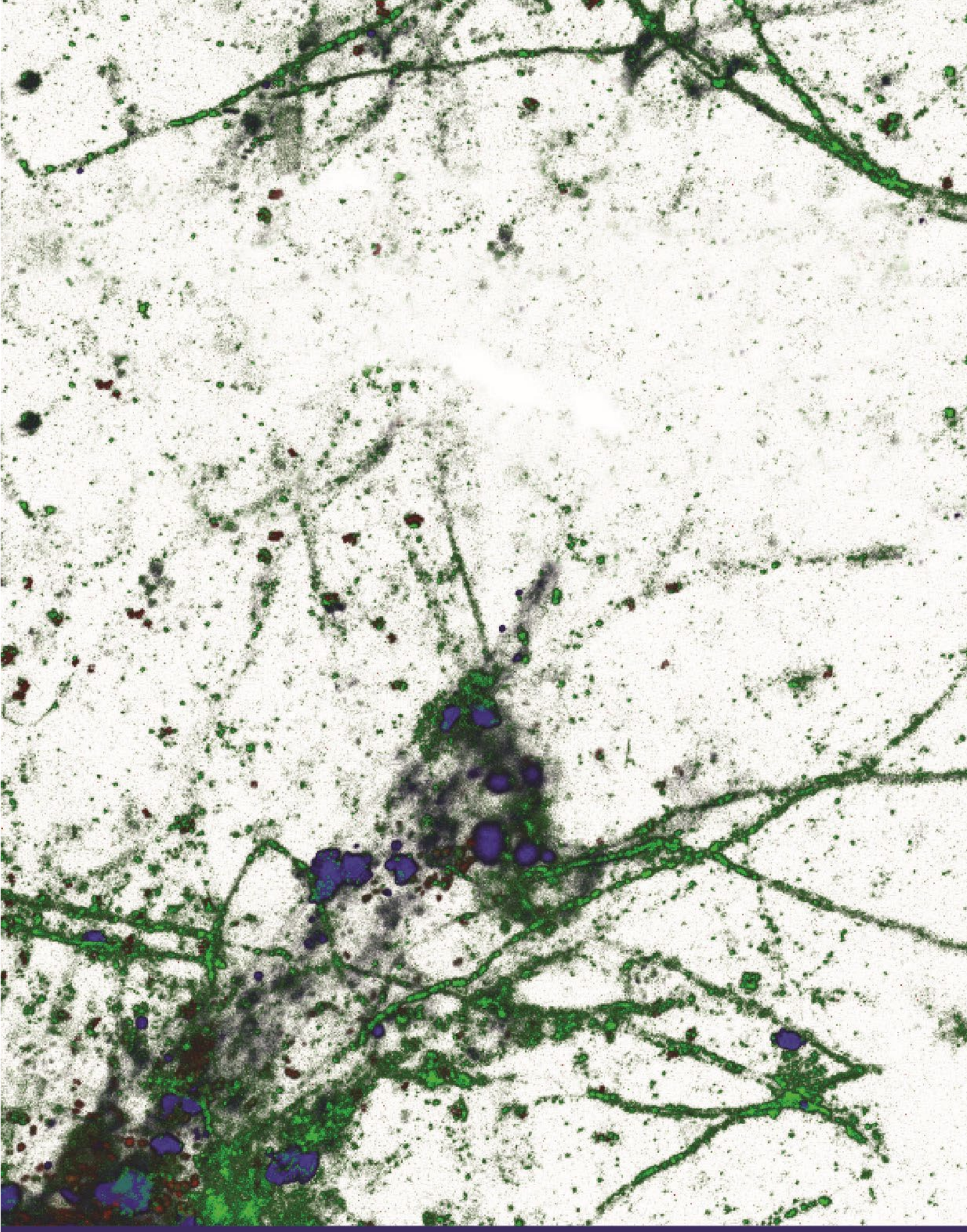
Jerica Sabotič
Department of Biotechnology
Jožef Stefan Institute
Jamova cesta 39
Ljubljana

Petra Seliškar
Department of Pharmaceutical Chemistry
Faculty of Pharmacy
University of Ljubljana
Aškerčeva ulica 7
Ljubljana

Marija Vukomanović
Advanced Materials Department
Jožef Stefan Institute
Jamova cesta 39
Ljubljana

Nika Zaveršek
Department of Biotechnology
Jožef Stefan Institute
Jamova cesta 39
Ljubljana

Martina Žabčić
Advanced Materials Department
Jožef Stefan Institute
Jamova cesta 39
Ljubljana



Institut
"Jožef Stefan"
Ljubljana, Slovenija