Latvian Institute of Organic Synthesis

Research strategy 2022-2027

Latvian Institute of Organic Synthesis

Contents

Abbreviations

ADME	Absorption, Distribution, Metabolism and Excretion
API	Active Pharmaceutical Ingredient
BSA	Bovine Serum Albumin
CAGR	Compound Annual Growth Rate
CMC	Chemistry, Manufacturing and Controls
CRO	Contract Research Organization
CYP	Cytochrome P450
DMPK	Drug Metabolism Pharmacokinetic
DSC	Differential Scanning Calorimetry
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practice
GPx	Glutathione Peroxidase
H2L	Hit to Lead
ITC	Isothermal Titration Calorimetry
L2CD	Lead to Candidate Drug
LC-MS	Liquid Chromatography - Mass Spectrometry
Met ID	Metabolite Identification
MST	Microscale Thermophoresis
MTD	Maximum Tolerated Dose
NIR	Near-Infrared
NMR	Nuclear Magnetic Resonance
NTSC	National Television Standards Committee
PCR	Polymerase Chain Reaction
PET	Positron Emission Tomography
PK/PD	Pharmacokinetic/Pharmacodynamic
PPB	Plasma Protein Binding
PVA	Poly(Vinyl Alcohol)
SPR	Surface Plasmon Resonance

1 Introduction

Latvian Institute of Organic Synthesis (LIOS) was established in 1957 to develop new small molecule drugs and pesticides. From the time of its establishment, the quality and impact of the research performed by the Institute has been extraordinary. Today LIOS is proud to be an inventor of 18 original medicines and more than 70 manufacturing processes of active pharmaceutical ingredients. The historical standards of scientific excellence at LIOS are maintained equally high today.

LIOS mission is to provide a contribution to the improvement of human health and the quality of life, including sustainability as an important condition for human well-being in a longer perspective. Accordingly, LIOS research is focused on two major strategic research areas: “Human Health” and “Sustainable Chemistry and Technologies” (**Figure 1**).

In the **human health** area, LIOS strategic goal is to address major societal health challenges as defined by United Nations and the European Union, where unmet medical needs exist. Accordingly, a drug discovery platform has been created at LIOS to provide a full research cycle necessary for the discovery of new candidate drugs (Section 2). Recent advances in material synthesis, analytical techniques and preclinical methods have put biomaterials into a leading position next to drugs in modern and future medicine. Therefore, biomaterials research, a relatively new research area at LIOS has been initiated to complement the LIOS drug discovery engine (Section 3). In addition, molecular diagnostics is playing an increasingly important role in preclinical and clinical studies. Thus, research aimed at the development of fluorescent probes as a tool for biomedical applications is expanding at LIOS (Section 4).

In the **sustainable chemistry and technology** area, LIOS research aims at minimizing the environmental impact of chemistry and reducing the consumption of nonrenewable resources. To this end, the design of a more resource-efficient and inherently safer design of chemical processes (Sections 5.1, 5.6) and materials (Section 7) is performed at LIOS. In addition, the transformation of biomass-derived renewable materials into high added value products has recently emerged a new research area at LIOS (Section 6).

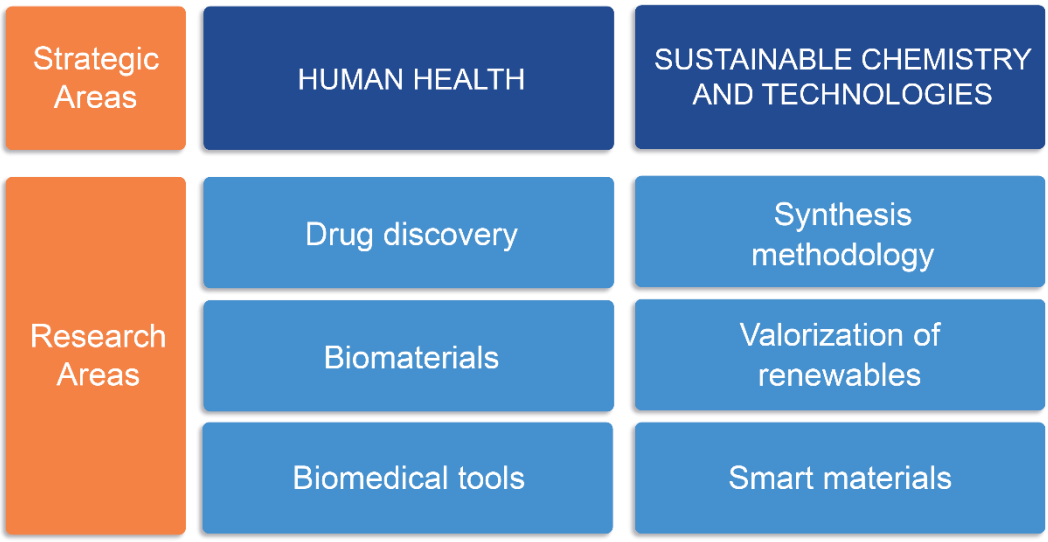

Figure 1. LIOS Research Areas

2 Drug design and discovery

2.1 Background

The major type of drugs discovered during the 20th century were small molecules. Small molecule drugs are mainly chemically synthesized compounds with a low molecular weight below 500 Da. Small molecules are able to affect the function of various proteins (enzymes, receptors), including protein–protein interactions, by forming complexes with these targets.[1] Small molecules represent approximately 90% of drugs on the market today and 75% of all new medicines approved in the US in 2020.[2] The advantages of small molecules include simple cost-effective manufacturing and administration routes, low specificity and a stable shelf life, meaning they are safe and effective for large groups of people. However, low specificity can also lead to side effects, reducing the chances of success in clinical trials. Since the 1990s, scientific and technological advances have led to the discovery of larger, more complex, biological therapeutics known as biologics. Biologics are medicines derived from living cells or through biological processes. They are relatively complex molecules mainly consisting of proteins, carbohydrates, nucleic acids or a complex composite of these substances.[3] For example, due to the complex manufacturing and administration routes only 17 of the 59 drugs approved by the Food and Drug Administration (FDA) in 2018 were biologics; during 2019 approved were 48 new drugs (38 new chemical entities and 10 biologics).[4] The 53 approvals in 2020 were divided between 40 new chemical entities and 13 biologic drugs (biologics).[5] Sixty percent of molecular entities approved in 2020 (32 approvals in total) utilized an orphan drug mechanism. Only one entity was formally approved for COVID-19 in 2020 – remdesivir. In 2020, the most prominent category for approved drugs remained oncology, with 21 molecular entities.[6]

In the past decade, major advances in the development of other types of drugs and therapies (biologics, cell and gene therapies) have put these novel treatment options in the spotlight. Thus, critical human health challenges are increasingly often addressed using biopharmaceuticals, which offer a range of benefits. This has resulted

1 Li, Q., Kang, C. Mechanisms of Action for Small Molecules Revealed by Structural Biology in Drug Discovery. *Int J Mol Sci.* **2020**, *21*, 5262. DOI:10.3390/ijms21155262

2 T.C. Mendonça Nogueira, M. V. N. de Souza New FDA oncology small molecule drugs approvals in 2020: Mechanism of action and clinical applications, *Bioorg. Med. Chem*. **2021**, *46*, 116340. DOI: 10.1016/j.bmc.2021.116340.

3 Makurvet, F.D. Biologics vs. small molecules: Drug costs and patient access. *Med. Drug Discovery* **2021**, *9*, 100075. DOI: 10.1016/j.medidd.2020.100075.

4 B.G.de la Torre, F. Albericio The Pharmaceutical Industry in 2019. An Analysis of FDA Drug Approvals from the Perspective of Molecules. *Molecules* **2020**, *25*, 745. DOI: 10.3390/molecules25030745.

5 B.G.de la Torre, F. Albericio The Pharmaceutical Industry in 2020. An Analysis of FDA Drug Approvals from the Perspective of Molecules. *Molecules* **2021**, 26, 627. DOI: 10.3390/molecules26030627.

6 M.S. Kinch, Z. Kraft, T. Schwartz, 2020 in review: FDA approvals of new medicines, *Drug Discovery Today* **2021**, *26*, 2794-2799. DOI: 10.1016/j.drudis.2021.07.003.

in a significant increase in the demand for biopharmaceutical products, and as of today, their market share has reached more than 25 percent of the total pharmaceutical market[7] and is forecasted to approach one third by 2026.[8] Despite these developments, small molecules have remained the most common treatment option and continue to play the most significant role in innovative drug development.

LIOS already has long-standing expertise in small molecule drug discovery and development for the treatment of cancer, cardiovascular, infectious diseases and CNS disorders (see Section 2.2 ”LIOS drug discovery platform”). This competence will be strengthened and expanded, particularly in the biopharmaceutical area to follow the global trend. In the meantime, LIOS will continue upgrading the already established drug discovery platform by introducing new competences/tools (see Section 2.3 “Therapeutic focus areas”).

2.2 LIOS drug discovery platform

According to the goals of the LIOS strategy 2016-2020, the institute has established a drug discovery and preclinical development (LIOS-DD) platform that consolidates the complementary expertise of LIOS research groups and the supporting infrastructure. The following structural units are involved in LIOS-DD: Medicinal and Process Chemistry groups and laboratories; Laboratory of Physical Organic Chemistry; Laboratory of Pharmaceutical Pharmacology; Biotechnology group. The integrated expertise of the platform consists of 3 major modules

(**Figure 2**)

1. Discovery of drug candidates;
2. Preclinical development;
3. Manufacturing technologies.

LIOS-DD can be applied to implement in-house and collaborative drug discovery projects as an integrated process from idea to candidate drug. Each of the modules or competences can also be applied separately depending on the project needs.

7 https://www.pharmamanufacturing.com/articles/2018/biopharma-market-an-inside-look/

8 https://www.statista.com/statistics/309450/pharma-revenues-worldwide-prescription-drug-and-otc-by-technology/

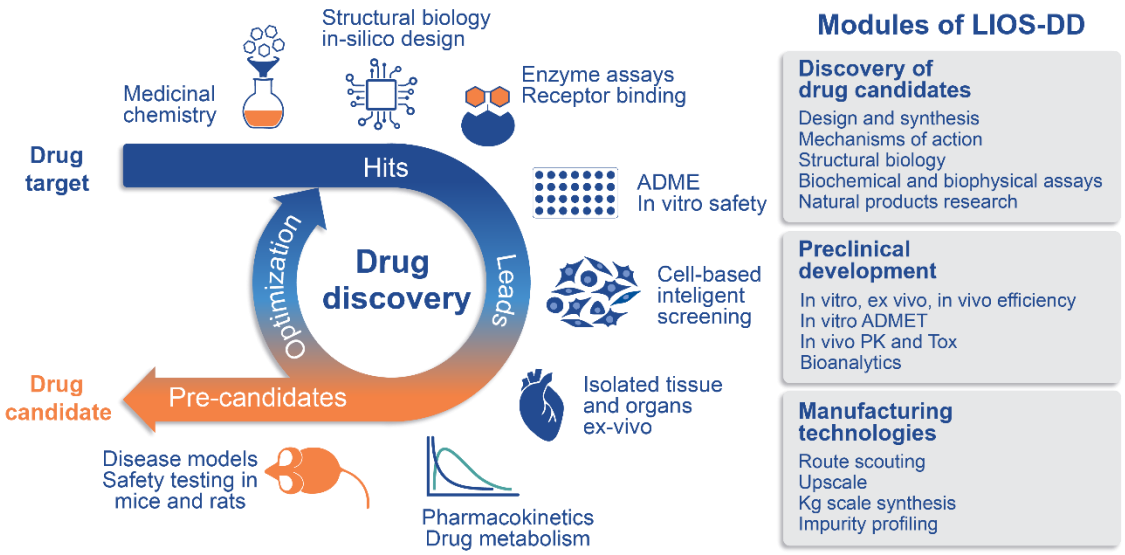

Figure 2. Drug design and discovery cycle of the LIOS-DD platform

2.2.1 Discovery of drug candidates

2.2.1.1 Drug target identification and validation

In most cases, new drug targets for LIOS medicinal chemistry projects are selected based on information available in scientific literature and public databases. In addition, target deconvolution is applied when the target is identified retrospectively for the efficacious drug or compound with a previously unknown mechanism of action. Additionally, the phenotypic approach is used both for drug discovery and target deconvolution studies. This involves exposing cells, isolated tissues, or animal models to compounds in order to determine whether a specific candidate molecule induces the desired phenotypic response. Target deconvolution is achieved by various methods, including enzyme and ion channel activity assays (in-house or external CRO), radioligand binding, and analysis of phenotype changes (metabolomics, transcriptomics). Basic research studies are performed to identify pathophysiological mechanisms and potential drug targets of a disease of interest. Drug targets are validated using disease-relevant cell culture and animal models. For the validation of target druggability, tool compounds and gene editing (e.g. knock-out) methods are applied.

2.2.1.2 Tools for drug discovery

- **Synthetic H2L and L2CD optimization programs** are performed by teams of highly skilled chemists collaborating with other experts implementing design-synthesis-testing rounds.
- ***In silico* design and virtual screening** are performed by computational modeling experts and supported by the Shrödinger software and CPU computer cluster.

- **Structure-based drug design** is assured by structural biology experts working in the field of protein NMR and protein X-ray crystallography.
- **Mechanism-based design** is used as an efficient strategy to develop leads by the collaboration of medicinal chemists with computational modeling experts
- **Bioisosteric replacements and scaffold hopping** have been proven to be very strong tools to reach the drug like properties when the erudition of medicinal chemists is combined with the expertise of computational modeling.
- **Natural product-inspired lead discovery** relies on the expertise of chemists performing the total- and the semi-synthesis of natural products. Notable achievements have been made in the development of natural product derivatives possessing anticancer, aphrodisiac, and CNS activities. Other research directions with distinguished track records are ethnopharmacology and the investigation of botanic products.
- **Fragment based lead discovery (FBLD)** is supported by biophysical methods such as NMR, SPR, ITC, MST and X-ray and the availability of four in-house fragment libraries. LIOS is involved in the H2020 project DRIVE as a WP leader devoted to establishing FBLD as a part of EU-OPENSCREEN services which has helped to expand the LIOS network.
- **High Throughput Screening (HTS) through partnership with EU OPENSCREEN** provides the network to collaborate medicinal chemists of LIOS with leading EU research centres performing HTS. An example of such a collaboration is project DRIVE, where LIOS provides med chem expertise for the development of hits resulting from HTS screening campaigns.

2.2.2 Preclinical development

2.2.2.1 ADME, DMPK and safety testing

The most effective compounds are passed to cell culture and *ex vivo* tests. In the final stage of development, *in vivo* tests are applied for preclinical studies. These are aided by initial ADME-Tox studies, including pharmacokinetics, the identification of metabolites and toxicity assessments (**Figure 3**)

Prior to actual dosing in animals, a number of relatively rapid and cost-effective *in vitro* assays can serve as surrogates and indicators of the ADME fate of compounds *in vivo*. Evaluation of compound ADME properties *in vitro* helps to predict their DMPK properties *in vivo*. To enable further compound analysis in various biological matrices for *in vitro* and *in vivo* tests, LC-MS/MS analysis methods for all prospective compounds/products have been developed. In an *in vitro* screening, it is possible to select compounds that are rapidly absorbed, well distributed, minimally metabolically degraded and not rapidly eliminated while not being toxic. Then, it is more likely to rapidly achieve peak levels in the blood, maintain the desired levels for a longer duration, before falling to low trough levels, and ultimately be cleared by the body. For in vivo PK studies in animals, such as mice and rats are employed to generate *in vivo*

PK data. Compound exposure (pharmacokinetic and toxicokinetic profile) is determined in parallel to efficacy and safety testing to determine exposure over a wide dose range. The nonclinical safety assessment of pharmaceuticals includes general toxicity studies and toxicokinetic studies, which include single-dose tolerability, MTD and repeated-dose toxicity investigations in rodents (**Figure 3**).

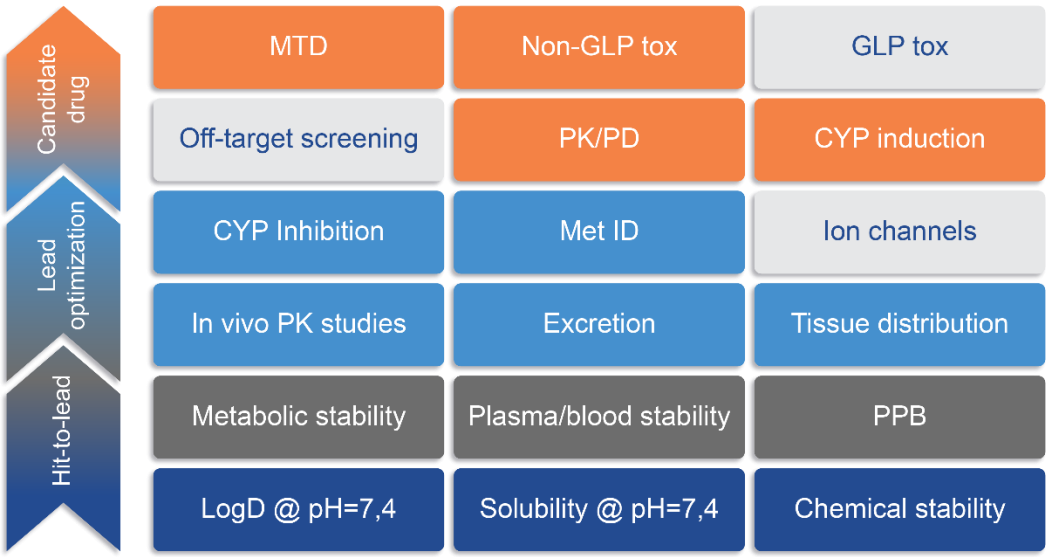

Figure 3. Preclinical ADMET profiling workflow and capacities at LIOS. The tests in white boxes are NOT performed at LIOS

2.2.2.2 Efficacy testing

The unit of pharmacological studies occupies 800m^2 of laboratory rooms with equipment for both *ex vivo* and *in vivo* testing of novel compounds. The animal facility fully corresponds to EU regulations of animal care. Experimental animals are obtained from certified local and international animal breathers. All animal care and experimental procedures complied with the local laws and policies and were approved by the Latvian Animal Protection Ethical Committee, Food and Veterinary Service, Riga, Latvia.

In vivo studies are an important part of the modern process of drug discovery. The LIOS unit of pharmacological studies is already using a wide range of *in vivo* disease models (**Figure 4**). In the future, it is planned to adopt new disease models. The main focus will be on acquiring new cancer models in mice. To promote the quality and validity of research data, we plan to participate in the preclinical randomized controlled multicenter trials of novel drugs and work according to the highest quality standards of the experimental model practices.

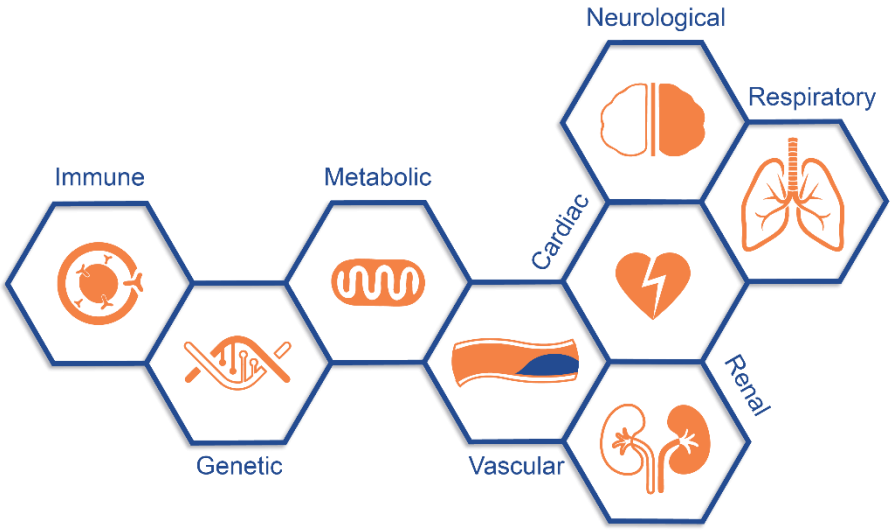

Figure 4. Disease models available at LIOS

2.2.3 Manufacturing technologies

LIOS has established competence and infrastructure to provide the CMC activities of the drug discovery and development process. These include a) process chemistry starting from route scouting to end-up with kg-scale production of API; b) analytical chemistry support including in-process control; impurity profiling and control together with GMP validation of analytical method; study of chemical stability, polymorphism and GMP stability control (**Figure 5**). The CMC work is applied for the production of preclinical batches of candidate drugs as well as for developing manufacturing technologies for original and generic drugs. A representative example for the use of CMC capacity is the support for scale-up work in IMI projects ENABLE and ERA4TB. It has been also applied for many collaboration projects with local and foreign industry partners.

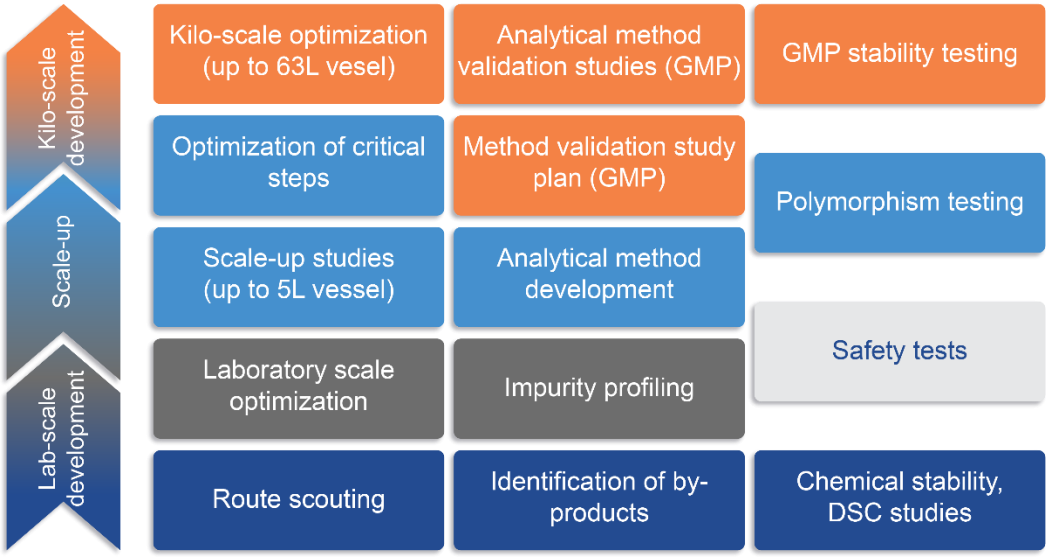

Figure 5. Route scouting and development workflow and capacities of LIOS. The tests in white boxes are NOT performed at LIOS.

2.2.4 Future strategy

The goal for the period 2022-2027 is to complement the existing LIOS drug discovery and development platform with new competences as well as to expand the existing ones. The upgrade is aimed both for more efficient small molecule drug discovery and development, and also for the development of biopharmaceuticals as a fundamentally new field in the LIOS research landscape. This would have an impact on all therapeutic focus areas (see section 2.3 below), substantially improving the capabilities and the performance. These improvements should also make LIOS more attractive as a collaboration partner for industry. Another important development aspect of LIOS-DD is the extension of capabilities from early preclinical investigations to clinical phase I according to the institutional strategy 2022-2027 (strategic working direction 1.2., action 1.1.7). This would require a set of competencies, infrastructure, and the implementation of quality control requirements, many of which would not be feasible at LIOS due to missing investments or going beyond the scope of an academic research organization. To fill these gaps, networking with other academic and nonacademic organizations is seen as the most efficient solution.

The following new competences/tools are to be developed to upgrade the LIOS-DD platform:

- The structural biology capabilities shall be expanded to include most modern methods, such as **cryo-electron microscopy** (cryo-EM) and **hydrogen-deuterium exchange** (HDX) mass spectrometry;
- A new research strand in **gene synthesis and formulation** shall be established in the context of vaccine development;
- Research in **bioconjugation methods** development shall be significantly expanded to address current problems in drug conjugate and therapeutic protein discovery;
- Design and synthesis of **peptide nucleic acids** as a tool for molecular biology and as potential drugs for RNA–related diseases shall be continued;
- Expand preclinical ADMET & PK studies (large animals, modern imaging methods, artificial intelligence methods);
- The biotechnology capabilities shall be expanded to include protein production in **eukaryotic expression systems**, which should open possibilities for in-house production of a wider range of our target proteins;
- In line with the expansion of the biopharmaceutical research direction, analytical methods for **biopharmaceutical product characterization** (attribute and stability assessment) should be established;
- Expanding **natural product research** to access natural products of pharmacologic relevance. Biosynthesis together with genetic engineering,

genome mining and exploiting unexplored natural product sources are useful complementary competencies;

- Introducing **new technologies for small molecule drug action** would be in line with growing trends to find alternative solutions to selectively target a protein/nucleic acid involved in disease pathology. Apart from bioconjugation (related to biopharmaceuticals), competencies in photopharmacology and PROTACS (protein degrading chimeras) would be a useful amendment to LIOS toolbox, in particular for an application to anti-cancer drug discovery;
- **Artificial intelligence-assisted drug discovery** (**AIDD**) is at its onset and will play a significant role given the advances in digitalization, data mining and machine learning. Introduction of this competence in LIOS-DD would complement the existing computer-assisted drug discovery and speed up the discovery process;
- Expanding the network for LIOS manufacturing technologies by **establishing collaboration with GMP** producers. This would enable more efficient transfer of chemical and analytical processes for the production of GMP batches of APIs.

By integrating these newly gained competences with existing expertise, LIOS shall be in position to undertake its own innovative small molecule and biopharmaceutical drug discovery programs as well as to offer specific expertise or services to collaboration partners and industry.

2.3 Therapeutic focus areas

2.3.1 Antitumor agents

2.3.1.1 Background

Cancers of different localizations are widespread and are a major cause of mortality of all ages. According to the International Health Organization, more than 7 million people diagnosed with various forms of cancer die every year. Tumour occurrence and progression are dependent on the certain cancer nature and the state of immunological reactivity. It determines the diversity of cancer therapy approaches that include surgery, radiotherapy, chemotherapy and immunotherapy to minimize the mass of the tumour.

Chemotherapy is the most often used treatment of cancers. Unfortunately, most chemotherapeutic agents cause damage to healthy cells, thus leading to severe side effects. In addition, the development of multidrug resistance is another major challenge to the success of existing chemotherapy treatments in cancer patients. Hence, there persists a high unmet medical need for the development of cancer chemotherapy agents that feature considerably reduced systemic toxicity and undesired side effects.

The overwhelming **majority of metastatic solid cancers cannot be cured by current systemic chemotherapies.** Recent developments in the field of anticancer medicine have delivered new classes of treatment – immunotherapy, including cell-

based immunotherapies, immunomodulators, oncolytic viruses, vaccines and monoclonal antibodies, the last class being the most widely used immunotherapeutic drugs globally. Immunotherapy is a type of treatment that uses the body's own immune system to attack cancer cells. The immune system works by attacking substances in the body it does not recognize. This includes viruses, bacteria, and cancer cells. Cancer cells present a major challenge during the use of therapy because they may not seem very different from normal cells to the immune system. Immunotherapy helps the immune system work better to fight cancer cells. The global cancer immunotherapy market accounted for $78 billion in 2019, and is estimated to reach $176 billion by 2025, growing at a CAGR of 14.5% during the analysis period.

However, neither method alone is able to eliminate all tumour cells and achieve a total recovery. The development of non-toxic antitumor drug candidates lies in the Unmet Medical Need category.

2.3.1.2 Research at LIOS

We have discovered a new lead compound (PA-27) for the suppression of breast and lung carcinoma and melanoma metastasis development with up to 100% efficacy. Notably, pre-treatment of laboratory animals with the compound led to the similar effect. The high efficacy was particularly encouraging, bearing in mind that PA-27 did not induce any major side effects. This unexpected discovery renders PA-27 a very promising immunomodulating drug candidate for the suppression of cancer metastasis development and prevention of tumour formation.

In addition, LIOS is developing a novel class of mitochondria-targeted anticancer compounds. Isoselenazolium salts selectively bind to cardiolipin (mitochondrial membrane-specific lipid), and by inhibiting pyruvate-dependent metabolism they induce dramatic increase in the production of reactive oxygen species in breast cancer cells (PMID: 33299068). Thus, isoselenazolium salts serve as a promising platform for the development of potent mitochondria-targeted drug candidates for anticancer therapy.

Finally, a series of patentable anticancer lead-like compounds that inhibit microtubule polymerization and exert antiproliferative activity with submicromolar growth inhibition values on multiple tumor cell lines have been developed at LIOS (ref. 11, section 2.3.1.4).

2.3.1.3 Future developments

Further developments will be aimed at drug candidates for targeted chemo- and immunotherapy with a good safety profile.

2.3.1.4 Key References

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2.3.2 Novel treatment for cardiometabolic disorders

2.3.2.1 Background

In the past 20 years, cardiovascular mortality has decreased in high-income countries in response to the adoption of preventive measures to reduce the burden of cardiovascular diseases and heart failure. Despite advancements in prevention and treatment, worldwide, sudden and unexpected cardiac death accounts for 17 million deaths every year. According to World Health Organization data, more than 422 million people worldwide have diabetes.[9] In addition, undiagnosed diabetes continues to be prevalent worldwide and in Latvia. Major complications of diabetes and cardiovascular diseases are the most common cause of death in the EU, resulting in the death of approximately 40%, or 2 million people a year.

2.3.2.2 Research at LIOS

LIOS has been developing drugs affecting mitochondrial functionality for over 40 years. In addition, close collaboration with the pharmaceutical industry (national-level partner JSC Grindex) has led to the possibility of translating the experimental discoveries into clinical practice. The cardioprotective drug, meldonium (trade name Mildronate), an efficient drug against cardiometabolic diseases, is one of the LIOS success stories. Discovered by the Institute, manufactured and marketed by Latvian pharmaceutical company JSC Grindeks, meldonium turnover exceeded 100 M EUR in 2020. The second-generation compound methyl-GBB phosphate (GX-EG2) completed phase I clinical trials in 2020.

The mechanism of meldonium and methyl-GBB mitochondria-targeted action is based on the decrease in long-chain acylcarnitine production in mitochondria (refs. 1 and 2, see section 2.3.2.4 below). This optimizes mitochondrial functionality and reduces the risk of acylcarnitine accumulation during ischemic events (refs. 2 and 3, section 2.3.2.4). Long-chain acylcarnitines affect the activity of several cytosolic and mitochondrial enzymes, ion channels, and signaling pathways. Long-chain acylcarnitines have been shown to decrease OXPHOS-dependent mitochondrial respiration and subsequently increase the production of reactive oxygen species (ref. 4, section 2.3.2.4). At physiologically relevant concentrations, palmitoylcarnitine was able to significantly decrease mitochondrial pyruvate and lactate oxidation (ref. 5, section 2.3.2.4) and impact cellular energy metabolism pathways through dephosphorylation of the insulin receptor and protein kinase B (Akt) (ref. 6, section 2.3.2.4). Pharmacological interventions that prevent acylcarnitine accumulation in various pathological states of mitochondrial dysfunction have been shown to reduce **myocardial infarction, atherosclerosis** and **diabetes** (refs. 1,7 and 8, section 2.3.2.4) and **cancer** (ref. 9, section 2.3.2.4). In addition to known targets (BBOX and

9 http://www.who.int/campaigns/world-health-day/2016/en/

OCTN2) for acylcarnitine decrease (refs. 10 and 11, section 2.3.2.4), recently novel target TMLD has been identified (ref. 12, section 2.3.2.4).

2.3.2.3 Major mitochondrial function-targeted drug discovery research goals in the future

- To discover novel druggable targets for the prevention or attenuation of cardiometabolic diseases;
- To identify the diseases or their complications that are linked with the accumulation of long-chain acylcarnitines;
- To synthesize and identify compounds that interact with mitochondrial drug targets;
- To choose and develop a new drug candidate for clinical development in collaboration with pharmaceutical industry partners.

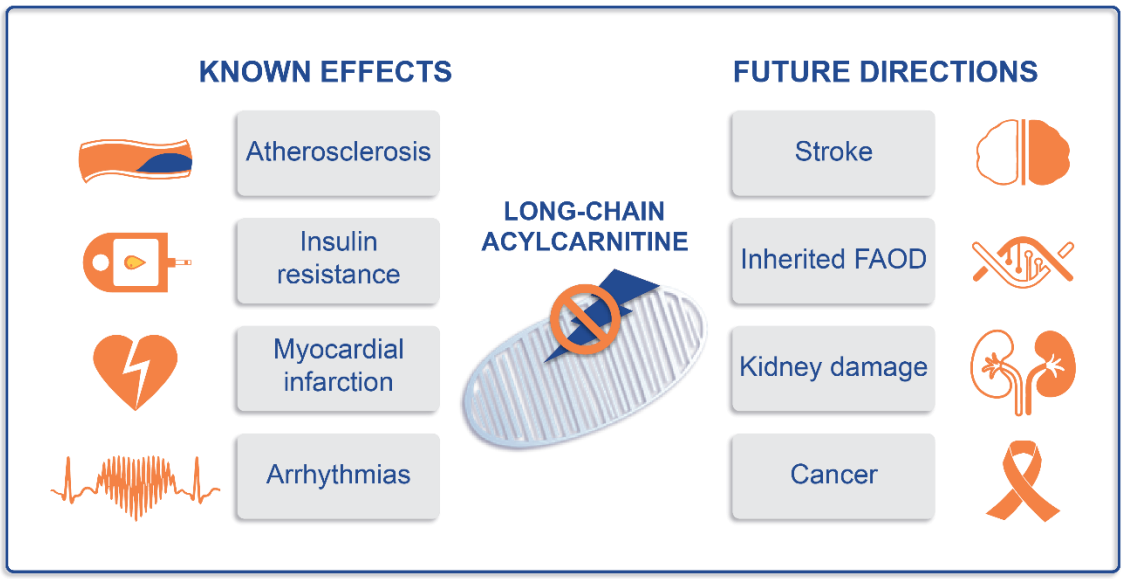

Figure 6. Long-chain acylcarnitine decrease was effective against many diseases in established preclinical models.

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2.3.3 Antimicrobial therapy

2.3.3.1 Background

Antimicrobial resistance (AMR) represents a serious and growing threat to human health worldwide. Every year 700 000 people die as a result of antibiotic-resistant infections, and the death toll could rise to 10 million by 2050. Despite an urgent need for new antibiotics, many large pharmaceutical companies have abandoned antibiotic research because antibiotic development is scientifically challenging and economically unprofitable.

2.3.3.2 Research at LIOS

Public–private partnerships have proven to be an efficient alternative to accelerate the development of new antibiotics. Since 2014 LIOS has served as a key partner in the antimicrobial drug discovery platform ENABLE (European Gram-Negative Antibacterial Engine), which was funded by the Innovative Medicines Initiative (IMI), a joint initiative between the EU and the European Federation of Pharmaceutical Industries and Associations (EFPIA), with the goal to replenish the pipeline of antibiotics for systemic Gram- negative infections, targeting the key pathogens *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. LIOS provided the medicinal chemistry and safety/pharmacology contribution to the platform, resulting in one phase I study completed, and five leads and three development candidates identified. The mechanism of action for the developed antibacterial molecules included inhibition of bacterial translation by peptides (ref. 6, section 2.3.3.4) and aminoglycosides (refs. 9, 10 and 11, section 2.3.3.4), inhibition of metallo-β-lactamases (ref. 12, section 2.3.3.4) and damaging plasma membrane (ref. 8, section 2.3.3.4).

2.3.3.3 Future Developments

A range of antimicrobial drug discovery projects are ongoing currently at LIOS:

- IMI2 ERA4TB, European Regimen Accelerator for Tuberculosis;
- Bacterial aaRS synthetase inhibitor development in collaboration with Oxford based Biotech;
- RANET-JPIAMR RESET-ME, Restoring E. coli Sensitivity for Antibiotics by blocking TolC-Mediated Efflux;

- ERANET-JPIAMR EXPLORE, Exploration of the TPP riboswitch as a new target for antibiotics;
- MSC-ITN CARTNET, Combating Antimicrobial Resistance Training NETwork;
- TEAMING project SPRINGBOARD.

Most of the above mentioned projects will be implemented beyond 2022, forming a large share of activities within the period 2022–2027. In addition, a new collaborative project ENABLE-2 is scheduled to start in 2022. It is expected that the expertise and the network established thus far will contribute significantly to engage in other projects that address the challenges of antimicrobial resistance.

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2.3.4 Antimalarial drug development

2.3.4.1 Background

Malaria, a disease caused by intracellular parasites of the genus *Plasmodium*, is a global health problem threatening more than half the Earth's population). Recent decades have seen a considerable reduction in the incidence of malaria and malaria-related mortality, largely due to the availability of efficacious chemotherapies and control of the mosquito vector. However, efforts towards malaria eradication are impeded by the alarming spread of drug-resistant parasites, rendering existing drugs ineffective in many regions. Of particular concern, resistance has now been reported to nearly all clinically used antimalarial drugs including artemisinins, the current front-line drug class. There is therefore an urgent need to bolster the antimalarial drug arsenal with new chemotherapeutics, particularly those with as yet unexploited mechanisms of action.

2.3.4.2 Research at LIOS

Research at LIOS has been devoted to developing antimalarial leads acting as malarial aspartic protease (plasmepsins), and subtilisin-like serine protease (PfSub1) inhibitors. A patented series of plasmepsin inhibitors (VAD-111) has entered preclinical studies including *in vivo* efficacy testing and ADMET profiling. Several generations of PfSub1 inhibitors have been developed to provide chemotypes showing activity in cell-based assays. This was proven to be a result of arresting the parasite egress from the red blood cells, validating PfSub1 as a target for antimalarial drug development.

Another target that is currently being investigated is Pf choline kinase. Intraerythrocyte growth of the parasite is accompanied by substantial biochemical and biophysical changes in the host cell membrane, featuring a dramatic increase in the phospholipid content, especially phosphatidylcholine and phosphatidylethanolamine, inside the infected erythrocyte. These are the major components of the parasite membrane, whose biosynthesis is a necessary requirement for the growth and replication of the parasite. Hence, targeting the parasite lipid metabolism constitutes an effective way of dealing with the spread of the disease and with conventional therapy-resistant strains.

2.3.4.3 Future developments

Building on the achieved results, the further research will be focused on inhibitors of aspartic protease (plasmepsins V, IX and X) and subtilisin-like serine protease (PfSub1), and threonyl trna synthetase inhibitors which have been validated as drug targets. Follow-up series will be generated to provide inhibitors with drug-like properties. A research project aimed at discovery of selective inhibitors constituting another direction for future work. Collaboration with world leading malaria biology experts (e.g. Prof. M. Blackman, Francis Crick Institute) would provide other antimalarial targets as medicinal chemistry objects.

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2.3.5 Treatment of neurological disorders

2.3.5.1 Background

Neurological disorders are the leading cause of disability and the second leading cause of death worldwide.[10] Neurological disorders are heterogeneous and involve complex pathological alterations with only a few effective treatments. The development of new therapies for neurological disorders such as epilepsy, stroke, depression, neuropathic pain, dementia, and Alzheimer's disease has the potential to improve the quality of life in patients, and reduce the future economic burden on healthcare systems. Despite recent advancements in *drug discovery*, the attrition rates due to lack of efficacy and *safety* issues remain high;[11] therefore, there is an urgent need for the development of new therapies for the treatment of neurological disorders.

2.3.5.2 Research at LIOS

LIOS has invented compound E1R ((4R,5S)-2-(5-methyl-2-oxo-4-phenyl-pyrrolidine-1-yl)-acetamide), which is a patented novel drug candidate for treating neurological disorders such as epilepsy and dementia (refs. 1,2 and 3, see section 2.3.5.4 below). These effects are attributed to its positive modulatory actions on the Sigma-1 receptor (Sig-1R). We showed for the first time that positive allosteric modulators of Sig-1R could be used as antiepileptic drugs. Moreover, we demonstrated that Sig-1R plays an important role in epileptogenesis and should be considered a promising molecular target for seizure modulation (refs. 4,5 and 6, section 2.3.5.4).

Another target that is currently studied at the LIOS is type 4 phosphodiesterase (PDE4). The neurological mechanism governing the learning and memory processes, depends critically on the cerebral levels of the second messenger cyclic adenosine monophosphate (cAMP) and on the correct functioning of the cAMP/PKA/CREB pathway. In the brain, cAMP levels are regulated by the activity of PDE4, an enzyme that hydrolyzes cAMP to 5′-AMP. Consistently, PDE4 is considered an important pharmaceutical target because of its crucial involvement in the signaling of the central nervous system. Indeed, some PDE4 inhibitors developed over time have been shown to improve memory and cognitive functions under both physiological and pathological conditions. Moreover, some PDE4Is are also used for the treatment of inflammatory diseases, such as respiratory disorders (COPD and asthma) and autoimmune diseases (rheumatoid arthritis, multiple sclerosis, and atopic dermatitis).

LIOS researchers have experience in the repurposing of old/existing drugs. For example, optically pure isomers of phenibut and phenylpiracetam were obtained and tested in various *in vivo* and *in vitro* tests (refs. 7 and 8, section 2.3.5.4). Moreover, we

10 Feigin V.L., Vos T., Nichols E., Owolabi M.O., Carroll M.D., Deuschl G., Parmar P., Brainin M., Murray C., The global burden of neurological disorders: translating evidence into policy. *Policy View* **2020**, *19*, 255-265.

11 Pangalos M.N., Schechter L.E., Hurko O. Drug development for CNS disorders: strategies for balancing risk and reducing attrition. *Nat. Rev. Drug Discovery* **2007**, 6, 521–532.

discovered new molecular mechanisms of action for the drugs, thus providing evidence of novel indications for their clinical use (refs. 9,10 and 11, section 2.3.5.4).

The studies of novel treatments for neurological diseases were organized in collaboration with national (JSC “Grindeks” and JSC “Olainfarm”) and international pharmaceutical companies, and funded by national and international academic research projects.

2.3.5.3 Future developments

- Continue a preclinical drug discovery for neurological disorders;
- To identify new drug targets for neurological disorders;
- To design multiple structurally diverse compounds for each target with a good safety profile.

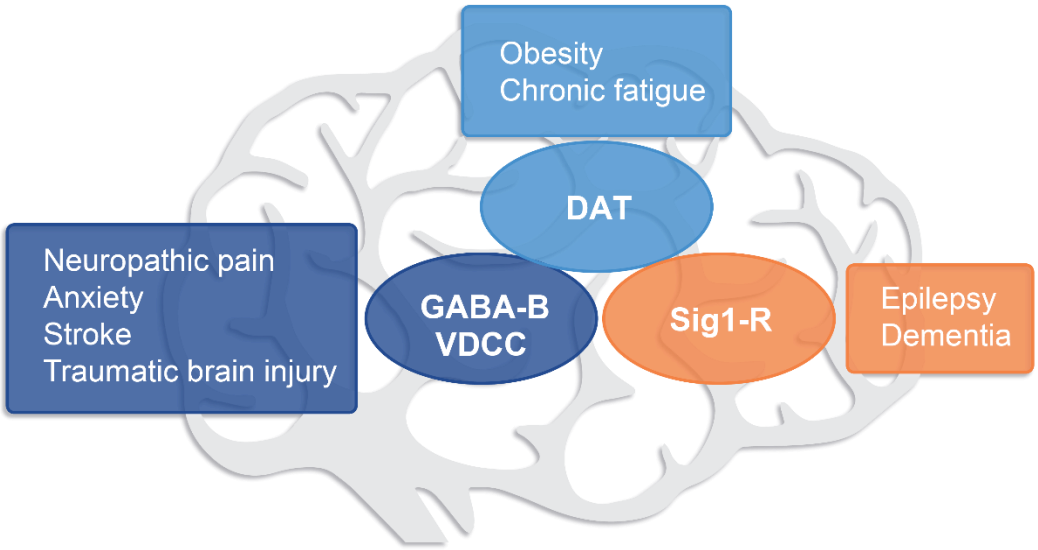

Figure 7. Development of drug candidates for neurological disorders.

2.3.5.4 Key References

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3 Biomaterials

3.1 Background

The term “biomaterials” has a broad definition, but biomaterials are generally understood as non-small-molecule substances that can be used for medical purposes (tissue and organ augmentation/replacement, therapy, diagnostics) or in biomedical research. Major advances in material synthesis, analytical techniques and preclinical methods have put biomaterials into a leading position in modern and future medicine.

Biohybrid systems stem from the combination of biomolecules and synthetic elements to give rise to new functions and applications such as chemical filtration, sensing, drug delivery and bioelectronics. The “biohybrid materials” research field, which has been growing tremendously over the last ten years, combines micro- and nanotechnologies with a biomimicry and bioinspired design.

According to the PubMed database, the number of publications related to bio- and biohybrid materials has increased two- and fivefold, respectively, over the last 10 years, and these numbers continue to increase steadily. Similarly, the expected CAGR for the biomaterials market is estimated at 13%. LIOS has capacity in three key aspects of biomaterial research: organic synthesis, structural and functional characterization and pharmacological evaluation. This capacity will be realized and expanded through the implementation of strategic directions described below.

Biomaterials research is a relatively new field at LIOS. Currently, three research groups are active in this area. The research is performed within the framework of Horizon2020 and European Structural Fund (ESF) projects in collaboration with the project partners. LIOS researchers contribute specific expertise to these projects (**Figure 8**).

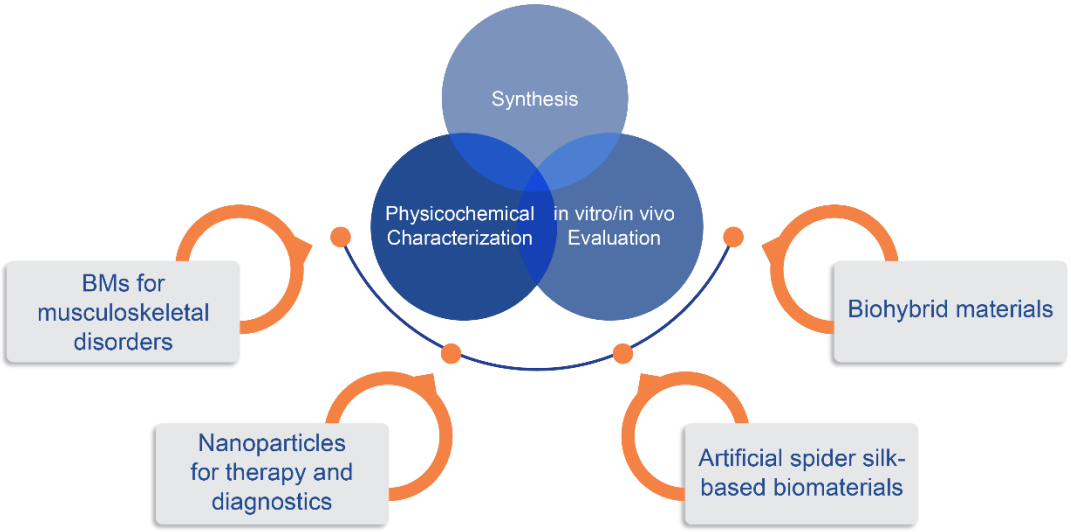

Figure 8. LIOS competence and research directions in the Biomaterials area.

3.2 Artificial spider silk-based biomaterials

3.2.1 Research at LIOS

Spider silk is a prospective biomaterial for applications in tissue engineering, artificial organ development and drug delivery. It combines high tensile strength and extensibility with lightweightness and biocompatibility. However, the production of artificial spider silk with natural-silk-like properties has been challenging due to our inability to reproduce the molecular mechanisms of native silk spinning. In collaboration with Karolinska Institute (groups of J. Johansson & A. Rising), LIOS researchers have studied the role of the spider silk protein (spidroin) terminal domains in the silk formation process (refs. 1-4, see section 3.2.3 below) and performed structural characterization of artificial fibres by solid-state NMR to understand the relationship between structure and mechanical properties (refs. 5 and 6, see section 3.2.3). These studies have allowed us to establish the mechanism of spider silk formation, which has several important implications for the spinning of artificial spider silk (ref. 7, section 3.2.3). The findings contributed to the development of the first biomimetic approach for artificial spider silk spinning (ref. 8, section 3.2.3). The solid-state NMR studies provided the first insights into the structure-mechanical property relationships of biomimetic artificial spider silk fibres. Furthermore, the identified properties of the spidroin N-terminal domain pointed to its potential application as a solubility tag for the efficient production of aggregation-prone proteins (refs 9 – 11, section 3.2.3).

3.2.2 Future developments

Despite significant progress over the last years, incompletely solved challenges remain in each step of the artificial spider silk production process. Therefore, a further increase in our understanding of the silk formation process and structure-property relationships is required to deliver the promise of spider silk. LIOS has the necessary expertise and equipment to contribute to these studies in several aspects. More specifically, we plan to address the following objectives:

3.2.2.1 Biotechnological production of spidroins

The recombinant expression yield of spidroins is important for developing a construct and technology that can be moved into production. To evaluate the expression yields from a bioreactor, we will seek collaboration partners to perform expression optimization studies with our most promising spidroin constructs using a medium-scale bioreactor.

3.2.2.2 Studies of flagelliform and tubuliform spider silk

Only one of seven types of spider silk - dragline silk - has been studied in great detail, while other silk types have received less attention. This has resulted in limited use of the other silk types, which display very different repetitive sequences and properties, for biomaterial development. To gain a more complete understanding of the amino acid sequence-recombinant protein expression efficiency relationship, silk formation mechanism and fiber structure-mechanical property relationships, we plan

to study two other spider silk types - flagelliform and tubuliform, using the approaches established with dragline silk.

3.2.2.3 Evaluation of effects of different fibre spinning and post-treatment conditions

Current research at LIOS is mainly aimed at the development of a novel method based on bioconjugation with polyethylene glycol for obtaining chemically modified (biohybrid) artificial spider silk in a biomimetic way. This approach could solve some of the challenges associated with the development of artificial spider silk-based biomaterials and reproduction of the molecular mechanisms of native spider silk spinning. It would also have several advantages in comparison to a purely biotechnological approach as it would ease recombinant spidroin production, allow cross linking between spidroins as well as easy tuning of material properties by varying the bioconjugation reaction conditions. As part of the project, we will create a silk spinning device based on a microfluidic chip and implement a method for testing the tensile strength of the fibres. This device will open new possibilities to study the structure-mechanical property relationships and determine the roles of specific domains or amino acid sequences in ensuring specific properties. Based on these findings, novel chimeric or engineered spidroins could be created with interesting properties not found in nature. Additionally, the device will enable in-depth studies of the impact of coagulation buffer composition and post-spinning treatments on fibre mechanical properties.

3.2.2.4 Understanding the role of silk fibre coating

The role of the coating in ensuring the high-performance properties of spider silk has not been studied in good detail. We plan to identify the components of the natural spider silk coat and investigate possibilities for coating artificial spider silk.

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3.3 Biohybrid materials

LIOS takes advantage of different international scientific collaborations in the field of biomedical materials characterization, with agreements related to the use of instruments in partner groups to minimize the replication of facility investment. At LIOS, the required instruments for the production of micro/nanofibers and particles

through the electrospinning and the electrospraying techniques are operational. This, together with the available molecular biology facilities, enables LIOS to produce biohybrid materials for the following fields of application:

3.3.1 Protein-decorated fibrous filters for chemical filtration and sensing applications.

We demonstrated that a biohybrid electrospun membrane consisting of BSA-loaded PVA fibers effectively removes ketoprofen from an aqueous solution by chemical filtration (ref. 1, section see 3.3.6 below). Starting from this proof-of-concept approach, we aim at the integration of these biohybrid systems in macroscale devices, which is a crucial step for any chemical filtration application. Moreover, these experiments provide a conceptual framework and an experimental platform for follow-up experiments in different technological settings, ranging from biosensing to probes and target immobilization for biotechnology, nanomaterial self-assembly, and environmental applications.

3.3.2 Immobilization of adhesion proteins to study cell adhesion mechanisms

Biofouling and bacterial colonization events occur because of the interaction of microorganisms and bacteria with the surface of almost all materials, including those used for implants and medical devices. The growth of bacteria into colonies, finally leading to biofilm formation, is a major threat to human and animal health and is one of the leading causes of the spreading of bacterial infections worldwide. By 2050, superbug infections are expected to become the leading cause of death. Hence, new strategies to fight antibiotic resistance are urgently needed. To this end, LIOS will adopt a multidisciplinary approach whereby material science, surface patterning and functionalization and drug design contribute synergistically to the inhibition of bacterial colonization, particularly in the context of nosocomial infections.

3.3.3 Study of the stability and activity of immobilized enzymes

Enzyme immobilization is one of the gold standards of the biotechnology field. Their immobilization on substrates has been proven to increase their stability and activity allowing a better tolerance of organic solvents or extending their pH working-range. LIOS will produce enzyme-loaded thin films and porous fibrous substrates following a “grafting onto” method where the enzymes are bound to a micro-structure or a non-covalent incorporation. The effect of this strategy on the stability and activity of the enzyme will be studied with chemical and biophysical approaches. At LIOS, the biohybrid materials research line will investigate this aspect in a synergistic effort with research groups, that are active in the field of enzyme engineering.

3.3.4 Effect of processing conditions on protein structure and activity

The increasing interest in the blending of biological elements with synthetic polymers for biohybrid material preparation has triggered the question of how delicate biological systems resist the challenging experimental conditions they are subjected to during technological processing. An exhaustive study of these effects on biologic

systems is still missing and LIOS possess all the chemical, biophysical and pharmacological expertise and equipment to shed light on this problem.

3.3.5 Photoactive biohybrid systems to photostimulate proteins and cells.

The material-cell interface is extremely important for *in vitro* studies and for implants. Nowadays, thanks to the integration of biotechnology and materials science, the development of smart interfaces is possible. By gifting the biohybrid material with luminescent properties, LIOS will study a smart scaffold triggered by an optical noninvasive input, which will allow mechanobiology and photopharmacological studies on cells and on tissue growth and development, a crucial step for biomaterial integration *in vivo*.

3.3.6 Key references

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3.4 Biomaterials for musculoskeletal disorders

LIOS is a partner in the Horizon2020 project “Baltic Biomaterial Centre of Excellence” or BBCE. The project is aimed at developing a strategic cooperation for advanced biomaterial development based on the long-term strategic cooperation between AO Research Institute Davos, Switzerland (ARI) and Friedrich-Alexander University of Erlangen-Nuremberg, Germany (FAU) on the one hand and LIOS, Riga Technical University (RTU), Riga Stradins University (RSU) and RSU Institute of Stomatology on the other hand. The BBCE project includes multiple short- and long-term visits to ARI and FAU, two world-leading organizations in biomaterial research for musculoskeletal disorders, for training purposes. This opportunity will be used to gain expertise in developing advanced models and workflows for biomaterials research related to orthopedics. Specifically, three strategic research areas will be pursued:

- methods for evaluation of biomaterials for substitution of tissues damaged by disease or trauma *in vitro*, including 2D cell culture of primary cells and development of organoids, and *ex vivo* explant cultures;
- medical implants to observe biomaterial performance *in vivo* and implants for localized drug release;
- biomaterials for drug delivery applications.

3.5 Nanoparticles for therapy and diagnostics

Nanomedicine is an emerging field that uses biomaterials to create novel diagnostics and therapies. Nanoparticles (NPs) have been extensively investigated for cancer therapy improvements, such as the reduction of systemic toxicity or the enhancement of drug accumulation in tumors as well as cancer diagnostics. Currently

there are efforts at LIOS to create nanoparticles that have minimal nonspecific interactions with the biological environment and that translate into exceptionally long circulation and body residence times. These NPs will serve as a tool to answer a longstanding question about the optimal NP size and to study nanoobject diffusion in healthy tissues and in tumors. With the help of LIOS expertise in synthesis, these NPs will further serve as a platform to create nanoparticles for cancer therapy and diagnostics.

Our strategic research areas are as follows:

- Development of polymers for biomedical applications;
- Creating nanoparticles with stealth-like properties;
- Develop methodology to study stealth nanoparticles of various sizes in many types of tumors;
- Use stealth nanoparticles and LIOS expertise in synthesis to create nanomedicines that can be used to deliver therapeutics locally and serve as cancer diagnostic tools.

3.6 Future strategy

The goal for the next period is to develop additional competences and build the capacity of the biomaterials research direction at LIOS. This will involve the development of infrastructure as well as the attraction of researchers. The desirable infrastructure improvements are:

- Size-exclusion chromatography system with refractive index, static light scattering, UV-Vis, viscosity, and fluorescence detectors;
- Dynamic scanning calorimeter;
- IVIS SpectrumCT or similar *in vivo* imaging system;
- Polymer thin-film processing machine (spin-coating);
- Biomaterials optical characterization microscopy (scanning electron microscopy).

Additionally, the involved research groups will seek synergies to extend LIOS contributions to the various projects and increase the impact of their research results. The specific objectives and research areas to be pursued during the next period are described in detail below.

3.7 Synergies

Several synergies between LIOS research groups working in the area of biomaterials are envisioned to help achieve the goals of the projects.

- Regarding polymer processing, LIOS possess all the necessary expertise to contribute to the understanding of the process of silk fiber formation and to the elucidation of the structure-property relationship in spider silk. In particular, the electrospinning technique, which is available on site, enables the production of

fiber mats with up to nanometric dimensions with precise control of the morphology of the fibers. This technique, which is widely used for the production of polymeric fibers, will be used with the different types of spider silk that are produced and engineered at LIOS, thus enabling an understanding of their processability. This will be followed by the characterization of the chemical structure, mechanical and biocompatibility properties of the spider silk-based fibers thus formed.

- As electrospun tissues well mimic biological tissues, they can be used as scaffolds for tissue engineering. This is due to the fine control that the technique allows on fiber porosity and arrangement on the final mat. Moreover, the possibility of electrospinning both natural and synthetic polymers enables the integration of electrospun mats within different natural tissues featuring different chemical and mechanical properties.

This approach will allow the study of (a) scaffolds for musculoskeletal disorders, and (b) spider silk-based electrospun tissues as biomaterials for tissue regeneration.

4 Biomedical tools

4.1 Background

The detection of signals in living organisms and the precise monitoring of pathological changes are of great importance for the early diagnosis of cancer, cardiovascular and infectious diseases, and other health disorders. The incessant rapid development of optical and photonic machineries provides various possibilities for optical detection and imaging, holding good opportunity for real-time visualization of biological signals in complex biological structures and processes. Notably, huge progress has been made thus far in the field of enzymes and DNA, and commonly used techniques such as PCR, microarrays, and *in situ* hybridization, are invaluable in understanding RNA processing and regulation. Antisense oligonucleotides (ASOs) have found broad application as biomolecular tools, molecular probes and biosensors. Sequence-selective recognition of nucleic acids by ASOs is achieved by the formation of Watson-Crick base pairs. On the other hand, most cellular RNA consists of noncoding RNAs (ncRNAs) that play important yet not fully understood roles in the regulation of gene expression. ncRNAs exist mainly in double-stranded conformations such as pseudoknots and hairpins, and the most selective and straightforward sequence readout for double-stranded RNA (dsRNA) would be the major groove triple helix formation (**Figure 9A**). However, for practice, triple helices are limited by the requirement for long homopurine tracts because pyrimidine nucleobases (Nb), unlike purine ones, can form only one hydrogen bond at the Hugsteen pair, and as a result, binding selectivity and stability are decreased.

Additionally, specific visualization of membrane function, especially mitochondria, in cells in general is still an unsolved task. Cardiolipin (CL) is a signature phospholipid of the inner mitochondrial membrane (IMM) in eukaryotes and the cytoplasmic membrane in prokaryotes. The latter is crucial for inward folding of the IMM and cristae formation. Additionally, CL directly interacts with electron transport chain complexes, promoting supercomplex formation, and is required for optimal activity of the respiratory chain. Similarly, anaerobic respiratory complexes in bacteria are stabilized by CL. Therefore, CL is essential for maintaining mitochondrial morphology and function. Distinctive structural properties and specific localization make CL an attractive pharmacological target for mitochondria-specific therapies, antimicrobial therapies and drug delivery strategies. Moreover, the mitochondrial toxicity of some drugs, e.g., anthracyclines and aminoglycosides, is in part attributed to their ability to interact with CL, leading to serious side effects such as heart failure and kidney damage. Notably, the level of CL also has clinical significance, as CL depletion is a hallmark of ischemic diseases, cardiac failure, diabetes, neurodegenerative disorders, and aging, and abnormally high CL levels and remodeling are identified as a novel molecular signature of thyroid oncocytic and prostate tumors.

4.2 Research at LIOS

4.2.1 PNA oligonucleotides for RNA-RNA-PNA triplex formation

Recently a new research direction aimed at sequence-selective recognition of dsRNA by peptide nucleic acids (PNAs) was initiated in collaboration with the Prof. Eriks Rozners group (SUNY Binghamton University, US). As a result of cooperative efforts, a new pyridazine-type Nb was found that selectively binds to cytosine and has at least two times higher affinity than those used to date. Another approach for pyrimidine Nb recognition is utilizing the entire Hoogsteen face of Watson-Crick base pairs in dsRNA. For this purpose, extended Nbs were developed that bind dsRNA in a quadruplex mode forming three hydrogen bonds. For example, single substitution of canonic timidine by amide group linked to pyrimidine-pyrazine Nb doubles stability of the PNA-dsRNA triplex (**Figure 9B**).

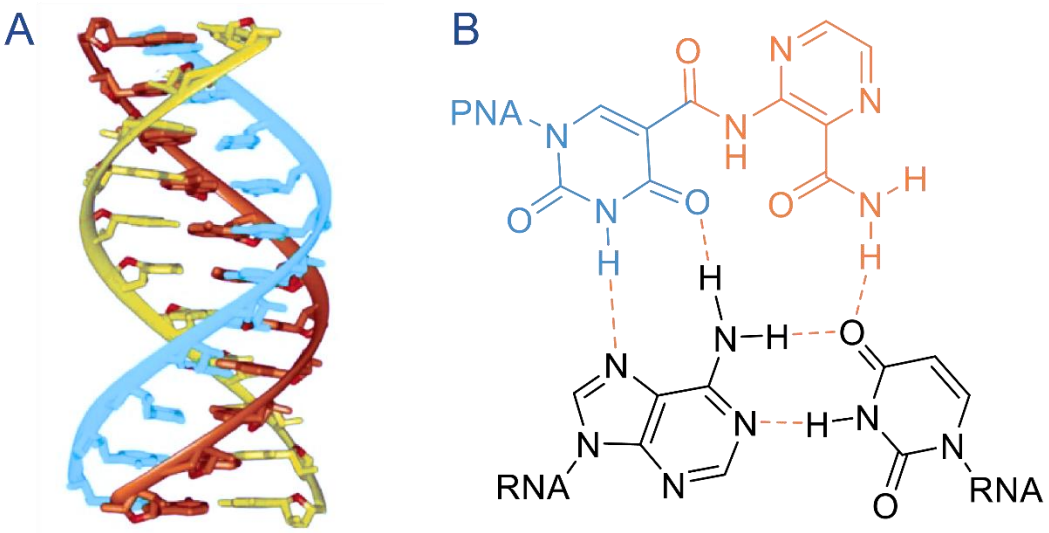

Figure 9. A: PNA-dsRNA triplex. **B:** Nb×A-U quadruplex.

4.2.2 Quantitative analysis of cardiolipin and fluorescence-based competitive binding assay

A new fluorescent CL-specific probe with impressive photophysical properties was developed that allows the estimation of drug affinity for CL in the first fluorescence-based competitive binding assay. Generally, positively charged substances bind with CL, but their affinity is highly variable, with EC_{50} values ranging from submicromolar to millimolar concentrations. The same probe can be used for CL quantification in mitochondrial fractions isolated from cell and tissue homogenates. The new screening technique allows us to evaluate CL as a potential molecular target for therapies designed to protect or damage the mitochondrial membrane, for antimicrobial therapy or targeted drug delivery into mitochondria.

4.2.3 Future developments

The long-term goal of our collaborative research will be to discover new PNA Nbs that will enable sequence specific recognition of any sequence of dsRNA. This

can lead to new tools for the imaging and functional control of RNA, designer riboswithes for synthetic biology and possibly to inhibitors of RNA-related diseases. Having in hands a convenient assay for quantitative analysis of cardiolipin and a competitive binding assay, we plan to deepen our knowledge of CL as a potential molecular target for therapies designed to protect or damage the mitochondrial membrane, for antimicrobial therapy, or as a landmark for targeted drug delivery into mitochondria. In addition, novel fluorescent probes will be developed in the near future, paying particular attention on NIR biomarkers.

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5 Synthesis Methodology

Synthesis methodology has been traditionally a key research area at LIOS, where LIOS has developed considerable expertise over the years. The considerable synthesis expertise at LIOS is an important prerequisite for conducting successful research in the Strategic Research areas – "Human Health" and "Sustainable chemistry and technologies". Clearly, the synthesis methodology plays an essential role in the development of new pharmaceuticals. Likewise, more effective use of materials as well as replacement of hazardous chemicals in an industrial process are focus areas of methodology research that can provide significant contribution to the main growth strategies of EU - mobilizing industry for a clean and circular economy or the EU Green deal.[12]

5.1 Catalysis

5.1.1 Cobalt-catalyzed C-H bond functionalization

5.1.1.1 Background

Over the past few decades, transition metal-catalyzed C-H activation has been immensely investigated due to the ability to functionalize relatively unreactive C-H bonds while simplifying synthetic schemes and making the synthetic pathway more economical. Recently, there has been a push to replace noble metal catalysts by the first-row transition metals that are earth-abundant, less toxic, and inexpensive. First-row transition metals (Fe, Co, Ni) have shown a rapid increase in inapplicability for C-H functionalization reactions, displaying comparable reactivity to precious metal (Pd, Ru, Rh) catalysts. In particular, simple cobalt(II) salts in combination with bidentate directing group chelation have been demonstrated to be efficient high valent Co(III) precursors. This approach has demonstrated great potential for $C(sp^2)$-H and $C(sp^3)$-H bond functionalization, allowing the construction of C-C, C-N, C-S, and C-Hal bonds.

5.1.1.2 Research at LIOS

Our work is dedicated to the development of a Co-catalyzed C-H functionalization methodology using directing group strategy. The main research directions are:

- Co-catalyzed C-H functionalization using weak, traceless, or transient directing groups;
- Meta-selective C-H functionalization.

5.1.1.3 Future developments

The research will be directed towards the development of novel Co-catalyzed C-H functionalization strategies. We will implement traceless, weak, or transient

12 Communication from the Commission to the European Parliament, the European Council, the Council, the European economic and social committee and the Committee of the Regions. The European Green Deal: COM/2019/640 final, EUR-Lex - 52019DC0640 - EN - EUR-Lex (europa.eu)

directing groups to achieve selective C-H activation in different types of substrates. Another research direction will be the development of meta-selective C-H functionalization using cobalt catalysis, which thus far has not been reported in the literature.

5.1.1.4 Key references

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5.1.2 Organocatalysis

5.1.2.1 Background

The demand for enantiomerically pure molecules in the pharmaceutical industry has promoted the development of numerous methods of stereoselective synthesis. Among them, enantioselective catalysis is especially attractive because it creates an optically active product from an achiral starting material. Furthermore, the use of catalysts is associated with a reduced environmental footprint as opposed to the use of stoichiometric amounts of chiral reagents. Catalysts for stereoselective synthesis are classified into three categories: enzyme-based catalysts, transition metal complexes, and organocatalysts (small organic molecule-based catalysts). Despite the fact that enzyme-catalyzed chemical transformations feature outstanding enantioselectivities, catalysis by enzymes is compromised by limited substrate scope, relatively narrow choice of solvents due to the poor solubility of enzymes, high cost of enzyme catalysts, complex procedures for their preparation, and laborious product isolation. The application of transition metal-based catalysts is also limited by their relative toxicity. Furthermore, the removal of trace transition metals from products is usually laborious, expensive, and produces large amounts of waste. These drawbacks have led to increased interest in the application of chiral, purely organic molecules as catalysts (organocatalysts) in the synthesis of enantiomerically pure materials. Organocatalysis has developed at an astounding speed since 2000, and the impact of organocatalysis on contemporary organic and pharmaceutical chemistry was recognized by the Nobel Prize in 2021.

5.1.2.2 Research at LIOS

The ongoing research at LIOS is focused on asymmetric Lewis-base catalysis by chiral DMAP and cyclic isothiourea species (such as HyperBTM). The main research directions are:

- The design of novel Lewis base catalysts and the development of their synthesis methods;
- The application of chiral Lewis bases in stereoselective catalysis

5.1.2.3 Future developments

The research plans comprise the design of chiral Lewis base catalysts for the development of stereoselective synthesis methodologies such as the dynamic kinetic resolution of cyanohydrins and amines, catalytic enantioselective cyclopropanation reaction, Steglish rearrangement, etc. Catalyst design and the development of synthetic methodologies will require an in-depth mechanistic understanding of respective stereoselective transformations, so the study of reaction kinetics and evaluation of a reaction profile by DFT methods will be performed for every catalytic process under development. The attraction of internal/local funding sources (LIOS internal grants for students, Latvian Science Council grants) is planned to accomplish the research. In addition, funding from pharmaceutical company Pfizer, Inc. (USA) within an ongoing collaboration program in the research topic will be also sought after.

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5.1.3 Photocatalysis

5.1.3.1 Background

The development of eco-friendly and cost-effective methods in synthetic organic chemistry has gained attention, as reflected by the increasing number of reports in the literature. Visible light photocatalysis is a rapidly emerging field with attractive advantages such as efficiency, sustainability, atom economy, and selectivity. Most organic molecules do not absorb visible light; therefore, the presence of photosensitizers such as transition metal complexes, organic dyes or semiconductors

facilitates the reaction. Photocatalysis is an efficient and sustainable route to achieve chemoselective reactions under mild and biocompatible conditions.

5.1.3.2 Research at LIOS

An efficient method for functionalization of short protected and unprotected selenocystine- peptides was developed using visible light-initiated reactions. The protocol is based on the generation of selenium radicals via visible light-initiated reactions in the presence of organic dyes. The selenium radical is further oxidized to an electrophile and trapped by *N*-heterocycles, providing respective Sec-containing indoles and azaindoles in good to high yields. Notably, intramolecular indole selenylation was performed providing Sec-containing indole-based macrocycles. Additionally, a straightforward method for the preparation of Se–S bond-containing peptides was developed utilizing visible light-initiated reactions. Significantly, unprotected peptides with “sensitive” amino acids showed excellent tolerance under the chosen conditions. Furthermore, we demonstrated that the Se–S bond-containing substrate is an oxidation-sensitive linker with potential application in biocompatible materials.

5.1.3.3 Future developments

It is planned to continue research in this direction for the development of eco-friendly synthetic methods. The main focus will lie on the elaboration of metal-free homogeneous & heterogeneous photocatalysis.

5.1.3.4 Key references

1. Lapcinska, S., Dimitrijevs, P., Arsenyan, P. Visible Light-Mediated Synthesis of Se−S Bond-Containing Peptides. *Adv. Synth. Catal.* **2021**, *363*, 3968-3972. DOI: 10.1002/adsc.202100751.
2. Lapcinska, S., Dimitrijevs, P., Lapcinskis, L., Arsenyan, P. Visible Light-Mediated Functionalization of Selenocystine-Containing Peptides. *Adv. Synth. Catal.* **2021**, *363*, 3318-3328. DOI: 10.1002/adsc.202100373.

5.2 Chemistry of hypervalent halides

5.2.1 Backgound

The chemistry of hypervalent iodine species has experienced tremendous development in recent decades, and hypervalent iodine(III) reagents developed by Koser, Stang, Zhdankin, and Togni have become mainstream reagents for a wide variety of synthetic transformations in contemporary organic synthesis. Notably, hypervalent bromine(III) species feature superior reactivity to their iodine(III) counterparts because of the higher oxidizing ability, stronger electrophilicity, and better leaving group ability (nucleofugality) of the bromanyl unit. Indeed, several unprecedented synthetic transformations have been already developed to demonstrate the remarkable synthetic potential of bromine(III) species. In spite of these notable accomplishments, the hypervalent bromine chemistry appears to be significantly less developed as compared to that of iodine(III) compounds. This striking

bias is to be attributed to the apparent lack of convenient synthesis methods to access bromine(III) species.

5.2.2 Research at LIOS

Ongoing research at LIOS is focused on the development of hypervalent bromine(III) chemistry. The main research directions are:

- The development of the electrochemical synthesis of chelation-stabilized hypervalent bromine (III) reagents
- The application of electrochemically generated bromine(III) species in organic synthesis

5.2.3 Future developments

The research will be directed at the development of a safe, inexpensive, and scalable methodology for the electrochemical synthesis of chelation-stabilized bromine (III) reagents using both batch electrolysis in an undivided cell and flow electrosynthesis. The electrochemically generated bromine (III) reagents will be employed in the development of efficient synthetic transformations that would benefit from the unique properties of hypervalent bromine(III) species. The attraction of internal/local funding sources (LIOS internal grants for students, Latvian Science Council grants) is planned to accomplish the research.

5.2.4 Key references

1. Sokolovs, I., Mohebbati, N., Francke, R., Suna, E. Electrochemical Generation of Hypervalent Bromine(III) Compounds. *Angew. Chem. Int. Ed.* **2021**, *60*, 15832–15837. DOI: 10.1002/anie.202104677.
2. Koleda, O., Broese, T., Noetzel, J., Roemelt, M., Suna, E., Francke, R. Synthesis of Benzoxazoles Using Electrochemically Generated Hypervalent Iodine. *J. Org. Chem.* **2017**, *82*, 11669–11681. DOI: 10.1021/acs.joc.7b01686.

5.3 Fluoroorganics

5.3.1 Background

Organofluorine compounds are of immense importance in pharmaceutical, agrochemical, and material research and industries. This can be illustrated by the fact that almost a quarter of pharmaceutical drugs in the market contain at least one fluorine atom in their structure. There is an increasing trend that we will be more dependent on fluorinated organic compounds and materials in the foreseeable future. The literature survey of recent developments in fluorine chemistry identifies several trends and topics: 1) sustainable and medicinal chemistry-compatible reagents and methodologies in fluorine chemistry; 2) strategic disconnections and bond formations in fluorine chemistry; 3) overcoming the poor reactivity of inorganic fluorides; 4) light-driven reactions in fluorine chemistry; 5) electrochemical fluorination; 6) late-stage

fluorination compatible with radiolabeling and PET imaging; and 7) catalytic enantioselective fluorination and fluoroalkylation reactions.

5.3.2 Research at LIOS

The current research at LIOS involves the development of reagents and methodologies for strategic disconnections and bond formations in fluorine chemistry:

- New fluoromethylene transfer reagents;
- Strategic incorporation of one-fluorine-one-carbon containing building blocks.

5.3.3 Future developments

The research will be dedicated to the development of new fluoromethylene transfer reagents and methodologies enabling previously inaccessible retrosynthetic disconnections in fluorine chemistry. In addition, new expertise will be acquired to increase LIOS capacity in the area of fluorine chemistry, such as overcoming poor reactivity of inorganic fluorides, light-driven reactions in fluorine chemistry, electrochemical fluorination, and fluoroalkylation. The possible funding options for the development of this research direction are LIOS internal grants, Latvian Science Council grants, PostDoc Latvia and European Research Council grants.

5.3.4 Key references

1. Sperga, A., Melngaile, R., Kazia, A., Belyakov, S., Veliks, J. Optimized Monofluoromethylsulfonium Reagents for Fluoromethylene-Transfer Chemistry. *J. Org. Chem.* **2021**, *86*, 3196–3212. DOI: 10.1021/acs.joc.0c02561.
2. Melngaile, R., Sperga, A., Baldridge, K. K., Veliks, J. Diastereoselective Monofluorocyclopropanation Using Fluoromethylsulfonium Salts *Org. Lett.* **2019**, *21*, 7174–7178. DOI: 10.1021/acs.orglett.9b02867.
3. Veliks, J., Videja, M., Kinens, A., Bobrovs, R., Priede, M., Kuka, J. *trans*-Fluorine Effect in Cyclopropane: Diastereoselective Synthesis of Fluorocyclopropyl Cabozantinib Analogs. *ACS Med. Chem. Lett.* **2020**, *11*, 2146–2150. DOI: 10.1021/acsmedchemlett.0c00220.

5.4 Carbocation Chemistry

5.4.1 Background

Carbocations (carbenium and carbonium ions) provide rich chemistry by their reactions with nucleophiles, rearrangements, and elimination of electrofuges. Developing methods for efficient carbocation generation and controlling their reactivity and stereochemistry enables metal-free eco-friendly and safe processes. In addition, carbocations are widely exploited in protecting group chemistry with broad application for chemoselective synthetic transformations. Another prominent example of carbocation significance is the biosynthesis of terpenes and terpenoids from isoprene,

where they are key intermediates in skeletal rearrangements resulting in very broad chemical diversity.

5.4.2 Research at LIOS

Research at LIOS in the field of carbocation chemistry has been focused on the amination of allylic carbocations, exploring the reactivity of nonclassical cyclopropylmethyl cations (CPMs), and new methods for carbocation generation.

5.4.3 Future developments

To develop eco-friendly and economical synthesis methods as well as to provide new chemotypes for drug discovery, the research is planned in the following directions: a) alternative methods for carbenium ion formation e.g. electrochemical and photo redox; b) controlled chemical diversity provided by the reactions of nonclassical CPMs; c) application of carbenium ion reactions in the synthesis of complex terpenoids; and d) extension of carbenium ion chemistry to radical cation chemistry.

5.4.4 Key references

1. Skvorcova, M., Lukasevics, T. L., Jirgensons, A. Amination of Carbenium Ions Generated by Directed Protonolysis of Cyclopropane. *J. Org. Chem*., **2019**, *84*, 3780-3792 DOI: 10.1021/acs.joc.8b02576.
2. Skvorcova, M., Jirgensons, A. Amide-Group-Directed Protonolysis of Cyclopropane: An Approach to 2,2-Disubstituted Pyrrolidines. *Org. Lett.* **2017**, 19, 2478-2481 DOI: 10.1021/acs.orglett.7b00584.
3. Skvorcova, M., Jirgensons, A. Intramolecular cyclopropylmethylation via non-classical carbocations. *Org. Biomol. Chem*. **2017**, *15*, 6909-6912. DOI: 10.1039/C7OB01721A.
4. Grammatoglou, K., Bolsakova, J., Jirgensons, A. C-Quaternary alkynyl glycinols via the Ritter reaction of cobalt complexed alkynyl glycols. *RSC Adv*. **2017**, 7, 27530-27537 DOI: 10.1039/C7RA03965D.

5.5 Selenium chemistry

5.5.1 Background

The refinery production of selenium worldwide is approximately 2700 tons annually. Seventeen percent of it is used in nutrition and cosmetics, and the rest – in the production of various materials. Over the past 20 years, scientific studies have clearly demonstrated that selenium is an irreplaceable microelement with essential properties for human health. Selenium is an active component of GPx, which is an enzyme involved in cell redox homeostasis. Indeed, relationships between the level of selenium in the daily diet and the risks of developing various types of cancers have been established. In recent decades, the introduction of selenium into biologically active molecules has attracted increasing attention. Unfortunately, from thousands of synthesized and studied selenium- containing compounds, not a single molecule has been approved as a drug thus far.

5.5.2 Research at LIOS

In recent years, the ongoing research at LIOS in the field of selenium chemistry has focused on the utilization of electrophilic selenium in the synthesis of selenium-containing heterocycles through the *in situ* generation of Se(I), Se(II) and Se(IV) halides from elemental selenium or selenium(IV) oxide.

5.5.3 Future developments

The main research goal is the development of novel methodologies for the synthesis of selenium and tellurium containing aliphatic and aromatic compounds, e.g., selenophenes, polyaromatic hydrocarbons, isoselenazolium, and indolizinium salts with the aim of finding the right direction for future research in the elaboration of drug candidates and smart materials.

5.5.4 Key references

1. Lapcinska, S., Arsenyan, P. Selenocysteinyl electrophiles efficiently promote the formation of coumarin and quinolinone cores by 6-endo-dig cyclization. *New J. Chem.* **2021**, *45*, 16625-16634. DOI: 10.1039/D1NJ02633J.
2. Lapcinska, S., Arsenyan, P. Straightforward Functionalization of Sulfur-Containing Peptides via 5- and 6-endo-dig Cyclization Reactions. *Synthesis* **2021**, *53*, 1805-1820. DOI: 10.1055/a-1343-5607.
3. Lapcinska, S., Arsenyan, P. Selenocystine Peptides Performance in 5-endo-dig Reactions. *Eur. J. Org. Chem.* **2020**, 784-795. DOI: 10.1002/ejoc.201901548.
4. Arsenyan, P., Lapcinska, S., Ivanova, A., Vasiljeva, J. Peptide Functionalization Through the Generation of Selenocysteine Electrophile. *Eur. J. Org. Chem*., **2019**, 4951-4961. DOI: 10.1002/ejoc.201900907.

5.6 Electrosynthesis

5.6.1 Background

Electrosynthesis relies on electrical current as a remarkably cheap reagent for red-ox transformations to avoid the use of expensive, toxic, and dangerous oxidizing and reducing agents. As such it has demonstrated potential for the development of sustainable organic chemistry methods in line with the principles of green chemistry: the use of electrical current in synthesis leads to improved atom efficiency, decreased waste and byproduct formation, and reduced energy consumption and costs. Another useful application of electrosynthesis is the generation of reactive intermediates whose generation through classical chemical techniques is difficult. Electrosynthesis has seen tremendous development in the recent decade which is supported by the commercial availability of technical setups.

5.6.2 Research at LIOS

Research at LIOS in the field of electrosynthesis has been focused on electrochemical reactant activation, electrochemical carbocation generation, the

oxidative transformation of furane derivatives, and heterocycle synthesis by tandem cathodic nitro group reduction/cyclization.

5.6.3 Future developments

The research is planned in the following directions: a) application for the generation of carbenium ions and radical cations; b) application for the valorization of renewables; c) application for C-H and C-C bond oxidation; and d) the use of electric current as terminal oxidants in transition metal-catalyzed transformations.

5.6.4 Key references

1. Darzina, M., Lielpetere, A., Jirgensons, A. Torii-Type Electrosynthesis of α,β-Unsaturated Esters from Furfurylated Ethylene Glycols and Amino Alcohols. *Eur. J. Org. Chem*. **2021**, *29*, 4224-4228. DOI: 10.1002/ejoc.202100605.
2. Lielpetere, A., Jirgensons, A. Friedel–Crafts Alkylation with Carbenium Ions Generated by Electrochemical Oxidation of Stannylmethyl Ethers. *Eur. J. Org. Chem*. **2020**, *29*, 4510-4516. DOI: 10.1002/ejoc.202000568.
3. Lielpetere, A., Jirgensons, A. Carbenium ion formation by fragmentation of electrochemically generated oxonium ions. *Org. Biomol. Chem*. **2018**, *16*, 5094-5096. DOI: 10.1039/C8OB01339J.
4. Rodrigo, E., Baunis, H., Suna, E., Waldvogel, S., R. Simple and scalable electrochemical synthesis of 2,1-benzisoxazoles and quinoline N-oxides. *Chem. Commun.* **2019**, *55*, 12255-1225. DOI: 10.1039/C9CC06054E.

5.7 Methodology-driven Total Synthesis

5.7.1 Background

Natural products have been broadly acknowledged as a rich source of biologically important compounds resulting in the development of a vast number of widely used therapeutics. One of the major tasks of total synthesis is to provide sufficient amounts of natural products for further biological studies since the natural feedstock is often limited. In this aspect, it is essential to develop user-friendly, safe, and concise synthetic methodologies to obtain sufficient amounts of natural products for biological studies. Even more important, the developed synthetic routes have to possess modularity allowing access to libraries of natural product analogs enabling structure-activity relationship studies (SAR) as well as tuning the ADMET properties.

5.7.2 Research at LIOS

Considerable experience in natural product chemistry has been gained at LIOS over the past decade culminating in a number of unique total syntheses. Natural products from several classes have been successfully synthesized, including indole alkaloids (refs 1 and 2, section 5.7.4), pyrrolo[1,4]benzodiazepine (PBD) natural products (ref. 3, section 5.7.4) and terpenoids (ref. 4, section 5.7.4).

5.7.3 Future developments

Further research at LIOS will be performed to address these major challenges of natural product-related medicinal chemistry:

- Development of user-friendly, safe, and concise synthetic methodologies enabling obtaining sufficient amounts of the natural products for biological studies;
- Biological evaluation of the synthesized natural products and their analogs *via* internal/external collaborations.

The attraction of internal/local funding sources (LIOS internal grants for students, Latvian Science Council grants) is planned to accomplish the research.

5.7.4 Key references

1. Kazak, M., Priede, M., Shubin, K.: Bertrum, H. E., Poisson, J.-F., Suna, E. Stereodivergent Synthesis of Pseudotabersonine Alkaloids. *Org. Lett.* **2017**, *19*, 5356-5359. DOI: 10.1021/acs.orglett.7b02635.
2. Ūdris, N., Jaudzems, K., Smits, G. Total Synthesis of the Proposed Structure of Uncarialin A. *J. Org. Chem*. **2021**, *86*, 6927-6930. DOI: 10.1021/acs.joc.1c00324.
3. Sakaine, G., Smits, G. Modified Julia–Kocienski Reagents for a Stereoselective Introduction of Trisubstituted Double Bonds: A Formal Total Synthesis of Limazepine E and Barmumycin. *J. Org. Chem*. **2018**, *83*, 5323-5330. DOI: 10.1021/acs.joc.8b00643.
4. Stakanovs, G., Mishnev, A., Rasina, D., Jirgensons, A. A Concise Bioinspired Semisynthesis of Rumphellaones A–C and Their C-8 Epimers from β-Caryophyllene. *J. Nat. Prod*. **2020**, *83*, 2004-2009. DOI: 10.1021/acs.jnatprod.0c00403.

5.8 Synergy with other Research Areas at LIOS

The organic synthesis methodology possesses a considerable synergy with virtually all research areas at LIOS (see **Figure 10**):

- **Synergy with Drug Discovery**. Expertise in organic synthesis enables accessing structurally diverse screening compounds in a short period of time at H2L process. It is also essential for process chemistry in the development of synthesis routes both for the customer-owned molecules and the drug candidates discovered by LIOS, as it considerably increases the commercial value of the drug candidate.
- **Synergy with research areas "Biomaterials" and "Biomedical Probes" and "Smart materials".** Competence in organic chemistry also plays a key role at the early stages of the development of novel smart materials, biomedical probes, and biomaterials. This significantly accelerates the evolution process allowing the

obtained in a short period of time. In addition, thodologies enable selective late-stage modifications the physical properties of the obtained materials.

ch area "Valorization of renewables". Expertise in ology also plays a key role in the valorization of ne with one of the main EU growth strategies – the efficient methods for the transformation of sustainable into high added value products is the main driving force w synthetic methodologies.

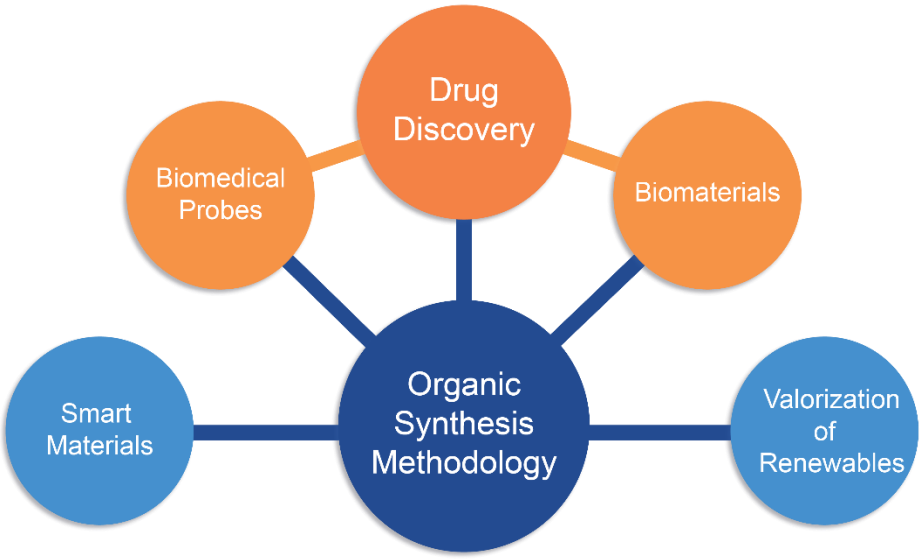

Figure 10. Synergy with other Research Areas at LIOS

6 Valorization of renewables

6.1 Background

The valorization of renewables is the utilization of biomass and urban waste to create the value-added products (**Figure 11**). Given the organic nature of the biomass and the large share of the urban waste, the organic chemistry and biotechnology play important role in this research area. The valorization of renewables can provide: a) novel chemotypes for an application in drug discovery and materials science; b) value-added products from already established biorefinery-produced platform chemicals as an alternative to fossil resources; and c) routes to new platform chemicals expanding the range of products produced from renewables.

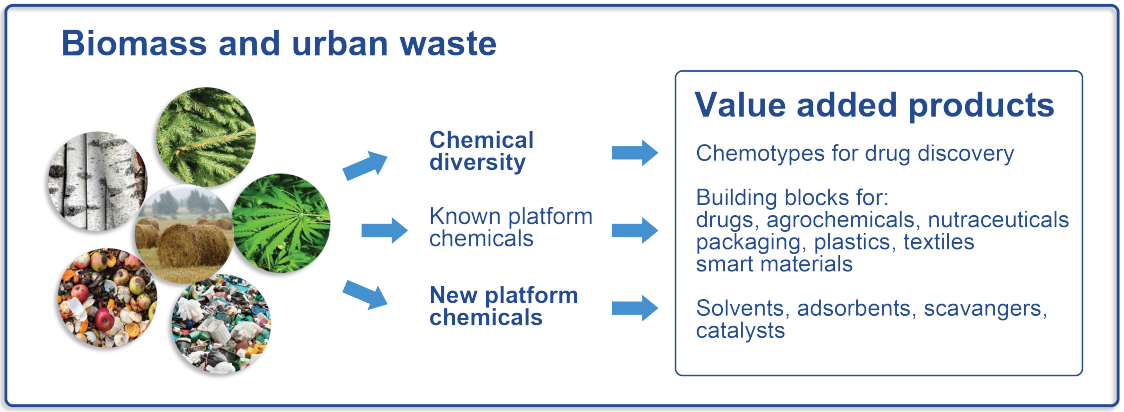

Figure 11. Valorization of renewables

The relevance of the valorization of renewable resources is in line with the Green Deal, a political initiative defined by the European Commission. This initiative is supported by establishing bioeconomy and circular economy strategies. One of the five goals of the bioeconomy strategy is to reduce reliance on nonrenewable and unsustainable resources (**Figure 12**).

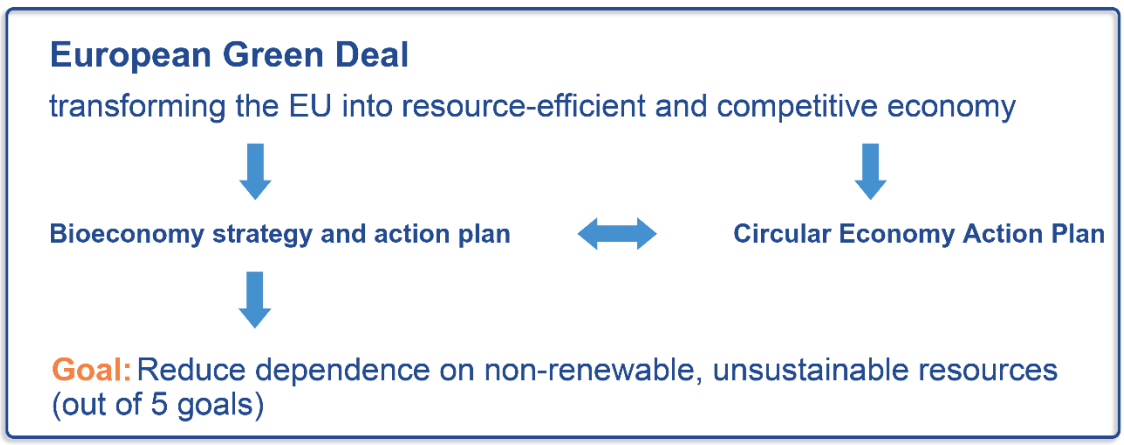

Figure 12. Valorization of renewables as a part of European Green Deal

Notably, the knowledge-based bioeconomy is one of the smart specialization areas identified in the research and innovation strategy for the transformation of the Latvian economy RIS3.

6.2 Research at LIOS

The field valorization of furfural, derived from biomass was central to LIOS activities in the 1950s and 1960s, culminating in the discovery of several furane-based drugs. However, this research direction has been neglected over the time and has seen only sporadic activities in recent decades. During the EU FP7 project "InnovaBalt", LIOS researcher D. Rasina visited the group of Prof. Vaccaro at the University of Perugia, where she worked on projects related to the application of biomass derived solvents for the organic synthesis. [13,14,15] LIOS researchers have recently published the electrochemical transformation of furfural derived substrates to acrylic acid derivatives (ref. 1, see section 6.5 below). Another research direction at LIOS is the valorization of caryophyllene as a nonexpensive abundant terpenoid. This has resulted in short semi-synthesis of low-abundance natural products (ref. 2, section 6.5). LIOS researchers have also engaged in a collaboration with SME regarding the construction of engineered enzymes for the use in the valorization of biomass.

6.3 Future developments

Organic chemistry and biotechnology expertise as well as an infrastructure holds the potential to expand the research area related to the valorization of renewables. The current activities at LIOS are relatively low, however they gradually increase. Fruitful directions in this area are: a) continued studies on the chemistry of abundant terpenoids, e.g. caryophyllene; b) focus on the chemical and biotechnological valorization of birch components such as oils, extractables, suberic acids, tannins etc.; c) utilization of components from spruce needles and leaves; and d) utilization of polymers *via* cooperative efforts of chemistry and biotechnology.

There are range of organizations, both academic and industrial, comprising the ecosystem of RIS3 - the knowledge-based bioeconomy. Some of them, such as Alternative plants, Latvijas Valsts Meži, Latvian Institute of Wood chemistry, and RTU Department of polymer chemistry are already a part of the LIOS network. The network

13 Rasina, D., Kahler-Quesada, A., Ziarelli, S., Warratz, S., Cao, H., Santoro, S., Ackermann, L., Vaccaro, L. Heterogeneous Palladium-Catalysed Catellani Reaction in Biomass-Derived γ-Valerolactone. *Green Chem.* **2016**, *18*, 5025. DOI: 10.1039/C6GC01393G.

14 Tian, X., Yang, F., Rasina, D., Bauer, M., Warratz, S., Ferlin, F., Vaccaro, L., Ackermann, L. C-H Arylations of 1,2,3-Triazoles by Reusable Heterogeneous Palladium Catalysts in Biomass-Derived γ-Valerolactone. *Chem. Commun.* **2016**, 52, 9777. DOI: 10.1039/C6CC03468C.

15 Rasina, D., Francesco, F., Santoro, S., Lombi, A., Vaccaro, L. Searching for Novel Reusable Biomass-Derived Solvents: Furfuryl Alcohol/Water Azeotrope as a Medium for Waste-Minimized Copper-Catalysed Azide–Alkyne Cycloaddition. *Green Chem.* **2016**, *18*, 6380. DOI: 10.1039/C6GC01941B.

in Europe and beyond is currently limited to the University of Perugia and Helmholz Institute and should be expanded for the application of EU projects.

6.4 Synergy with other LIOS research areas

The valorization of renewables has a strong synergy with the research area “Synthesis Methodology” as the newly developed methods can be applied for the chemical and electrochemical transformations of biomass and the urban waste. On the other hand, the need for specific degradation routes is a driver to develop new synthetic methods. The products of biomass and the urban waste transformation can find a useful application in the research area of drug discovery, providing unprecedented chemotypes as well as sustainable building blocks for drug manufacturing. The challenges of the research areas “Biomaterials” and “Smart Materials” provide the requirement for sustainable building blocks addressing the research activities of valorization of renewables.

6.5 Key references

1. Darzina, M., Lielpetere, A., Jirgensons, A. Torii-Type Electrosynthesis of α,β-Unsaturated Esters from Furfurylated Ethylene Glycols and Amino Alcohols. *Eur. J. Org. Chem*. **2021**, *29*, 4224-4228. DOI: 10.1002/ejoc.202100605.
2. Stakanovs, G., Mishnev, A., Rasina, D., Jirgensons, A. A Concise Bioinspired Semisynthesis of Rumphellaones A–C and Their C-8 Epimers from β-Caryophyllene. *J. Nat. Prod*. **2020**, *83*, 2004-2009. DOI: 10.1021/acs.jnatprod.0c00403.

7 Smart Materials

7.1 Background

In the last decade organic light-emitting diodes (OLEDs) and light-emitting electrochemical cells (LEECs) have become prevalent in display technology. The self- emitting ability, transparency, true dark tone, light weight, thinness, quick response time and flexible properties of OLED displays render them superior to liquid crystal displays. Despite great advances in improving the internal quantum efficiency of an OLED to nearly 100%, the external quantum efficiency is still lagging behind due to low efficiencies and short lifetimes. The advancement of OLEDs and LEECs requires rational design of light-emitting luminescent molecules (luminophores). Of note, OLEDs and LEECs are solid-state lighting devices that require the luminophores to operate in an aggregated state. In the aggregated state luminophores undergo intermolecular interactions that usually decrease their efficiency, leading to aggregation-caused quenching (ACQ). Hence, the development of emitting materials possessing high luminescence efficiency both in solution and amorphous/crystalline phases is of high importance. Recently, it was reported that thermally activated delayed fluorescence (TADF) emitters can be used for the fabrication of high-efficiency OLEDs. Such devices are fabricated utilizing pure organic materials that can acquire both singlet and triplet excitons for radiative transition. Therefore, TADF luminophores are appropriate as high-efficiency emitters for OLEDs and are replacing their fluorescent and phosphorescent counterparts.

7.2 Research at LIOS

7.2.1 Aggregation-induced emission (AIE) materials

In 2019, a new research project aimed at the development of LEECs and the design of purely organic luminophores that would feature very high emission intensity in the aggregated state was initiated at LIOS in collaboration with Institute of Solid State Physics (University of Latvia). High emission in the solid state was achieved by introducing pyridinium subunits in the luminophore structure to secure intermolecular π^+–π and π^+–π^+ interactions between luminophores. The π^+–π and π^+–π^+ interactions promote intermolecular charge-transfer type emission and help to avoid the ACQ, resulting in materials with 80% PLQY in the solid-state. In collaboration with researchers at the Institute of Solid State Physics, prototype LEECs were produced, and their electronic and thermal properties were examined. A prototype LEEC system features a maximum current efficiency of 6.08 cd/A. The use of the intermolecular π^+–π interactions has also helped to design a sensor that features emission turn-on upon protonation and is suitable for sensing hydrogen chloride and sulfonic acids (TfOH, TsOH and MsOH) with detection limits spanning the range from 0.06 to 0.33 ppm.

7.2.2 Development of new OLEDs

The research devoted to the design of new TADF and phosphorescent emitters was performed in collaboration with Prof. Juozas V. Grazulevicius laboratory at

Kaunas University of Technology. In the last 5 years, several achievements in the elaboration of prospective TADF emitters have been made. Pyridine-3,5-dicarbonitrile bearing *tert*-butyl-substituted carbazoles showed the best combination of TADF and charge-transporting properties (having 0.04 eV of ΔE_{ST} and 41% of PLQY in doped layers and achieving 2.8×10^{-3} $cm^2/(Vs)$ of hole and 1.2×10^{-4} $cm^2/(Vs)$ of electron mobilities at electric field of $5.2\cdot10^5$ V/cm in nondoped layers). Optimized doped devices showed maximum external quantum efficiencies (EQEs) of 7.5%. Substituted dicyanopyridines with three donors of one type (carbazole) or of two types (carbazole and phenothiazine) in their molecular structures are characterized by efficient green and orange TADF which results from recombination of two intramolecular charge transfer states. OLEDs based on the carbazolyl multiple substituted dicyanopyridine exhibiting normal TADF showed relatively high device lifetimes and high maximum external efficiencies of 8.1 (for nondoped device) and of 25% (for doped devices).

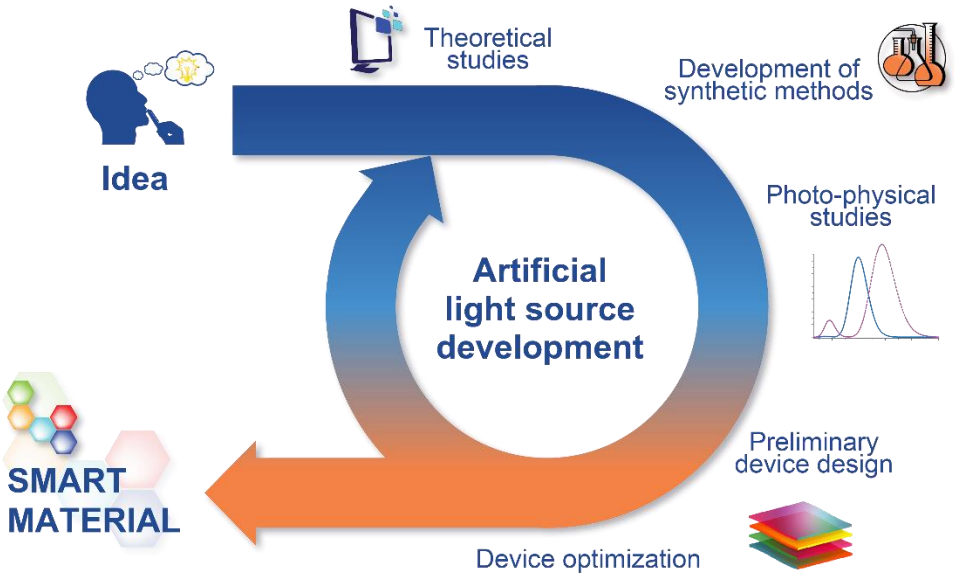

Figure 13. Workflow for the artificial light source development at LIOS.

Another research direction relates to the development of red emitters. A very limited number of efficient red phosphorescent emitters with CIE 1931 chromaticity coordinates (x, y) close to the NTSC red colour standard of (0.67, 0.33) is currently available. Overcoming this challenge may have a positive effect on the development of both deep-red phosphorescent organic light emitting diodes (PHOLEDs) and white OLEDs with a high colour rendering index (CRI) required for OLED displays and OLED lighting technologies, respectively. Selenium is very popular in LED technology, however, only a few examples have been found in the literature for the utilization of selenium in the production of OLEDs. Motivated by the idea to understand a possible impact of the introduction of this heavy element on the deep red PHOLEDs structure, we developed methodology for the synthesis of novel iridium(III), platinum(IV), and gold(III) complexes containing (benzo[*b*]selenophen-2-yl)pyridines as C^N ligands. Meticulous characterizations of the developed complexes proved their potential application as deep red PHOLED emitters. Thus, vacuum-deposited PHOLEDs based

on Ir(III) emitters exhibited deep red electrophosphorescence with even deeper chromaticity coordinates of (0.691, 0.309) than the corresponding NTSC standard. In addition, white hybrid OLEDs were developed using the newly synthesized selenium-containing deep-red iridium phosphorescent emitters. High-quality white electroluminescence with a colour-rendering index reaching of 85 was observed from the hybrid partly solution-processed OLEDs. The devices exhibited maximum brightness exceeding 10000 cd/m^2 and high EQE of 6.26% for solution-processed white PHOLEDs. The best fabricated deep red PHOLED showed maximum current, power and external quantum efficiencies of 5.2 cd/A, 2.1 lm/W, and 7.53%, respectively.

7.3 Future Developments

Future efforts will relate to the development of highly effective OLEDs and LEECs reaching CRI>85. A special task is to develop a new highly efficient and reliable triplet-triplet fusion (TTF) blue emitters and OLEDs with high efficiency and long operating lifetimes (up to 60,000 hrs). The detailed investigations will include the design, theoretical characterization, synthesis and identification of highly efficient blue organic fluorescent TTF emitters. The device design will consider the properties of the most promising new TTF emitters or device design to achieve the carrier balance, efficient exciton generation and energy transfer, and improved light out-coupling to reach EQE of over 15%. Additionally, we will focus on the development of highly efficient amorphous-state (>80% PLQY) pyridinium luminophores for incorporation into LEEC thin layers. This will be achieved by designing rigidly ''locked'' intramolecular π^+–π interactions within a luminophore, which will mitigate the emission loss in the amorphous state. Additionally, pyridinium luminophores that would feature fast (<1 s) turn-on times and long device lifetimes in LEECs will be designed. Furthermore, materials that feature emission from triplet states with long emission lifetimes (>200 ms) will be also addressed.

7.4 Key references

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