Description of the main research directions investigated by the institute

Bioorganic and medicinal chemistry

The team (Organic chemistry I, CHEM I) consists of several research groups of different types and status: Senior Research Groups of Prof. M. Hocek, Dr. Z. Janeba and Dr. R. Nencka, Junior Groups of Dr. M. Vrábel and Dr. D. Yushchenko, Research Service Group of Dr. P. Majer, and Targeted Research Groups of Dr. E. Kudová and Dr. D. Rejman, and Distinguished Emeritus group of Dr. I. Rosenberg. Each group has their own independent research programme with different ratio of basic science and applied research depending on the type of the group.

The Hocek group works in bioorganic and medicinal chemistry of nucleosides, nucleotides and nucleic acids. The medicinal chemistry programme deals with the design, synthesis and biological profiling of base-modified nucleosides targeted for cytostatic or antiviral activities. In the bioorganic chemistry, the Hocek group developed synthetic methodology for direct modification of nucleoside triphosphates and enzymatic methods for syntheses of modified nucleic acids for diverse applications in bioanalysis, diagnostics or imaging, as well as in chemical biology (chemical switches of transcription or cross-linking of nucleic acids with proteins). More info: http://hocekgroup.uochb.cas.cz/

The Janeba group is involved in the organic synthesis and medicinal chemistry of modified nucleoside and nucleotide analogues and other heterocyclic compounds. Medicinal chemistry oriented research deals with novel types of acyclic nucleoside phosphonates (and bisphosphonates) with potential antiviral, antibacterial, antiparasitic and anticancer activity and with polysubstituted pyrimidines with potent anti-inflammatory or antiviral properties. Synthetic methods are developed for the preparation of novel biologically active compounds for the medicinal applications and also for polysubstituted 5-nitrosopyrimidines and 5-phenylazopyrimidines with interesting physicochemical properties (e.g. potential molecular photoswitches). More info: https://janeba.group.uochb.cz/en

The Nencka group is focused on modern medicinal chemistry and drug design. One major goal is to develop therapeutic agents against selected human diseases. Another is to identify novel chemical tools that allow deciphering of pathological processes and enable development of potential treatments. The main topic of the group research has been antiviral drugs targeting both viral proteins and host factors, but recently the group have also utilized the experience in medicinal chemistry in several projects focusing on neurodegenerative diseases and metabolic disorders. More info: https://nencka.group.uochb.cz/en

The Vrábel group works on the development and application of various biocompatible chemical reactions. The developed tools are used for bioimaging applications to visualize and study biomolecules or biologically active small molecules in complex cellular environment. In addition, the group employs combinatorial libraries to produce bioconjugates with improved properties and functions. https://vrabel.group.uochb.cz/en

The Yushchenko group worked in chemical biology and medicinal chemistry. The chemical biology research includes creation of fluorescent and light-controllable tools for studies protein interactions. The medicinal chemistry work is focused on studies of Parkinson's disease related protein α-synuclein and development of inhibitors preventing its misfolding. https://yushchenko.group.uochb.cz/en

The Majer group (Drug Discovery) is active in the field of biologically active compounds. The group has extensive network of collaborations with biochemical and theoretical group within the IOCB. The other main effort is devoted to discovery of new medicines in collaboration with

Johns Hopkins University School of Medicine which provides the biological testing and actively searches for partners to further develop jointly patented technologies. https://drugdiscovery.group.uochb.cz/en

The Kudová group targets synthesis of novel neurosteroids as neuroprotective agents. The medicinal chemistry programme deals primarily with the design, synthesis and biological profiling of neurosteroids with principal focus on modulation of *N*-methyl-*D*-aspartate receptors. Inhibition or potentiation of these receptors can afford physiological and pathological outcome and as such, modulators of *N*-methyl-*D*-aspartate receptors can exhibit neuroprotective potential and minimal side effects in animal models of several neurological diseases like epilepsy, neuropathic pain, ischemia, neuropsychiatric disorders, and others. More info: https://kudova.group.uochb.cz/

The Rejman group works in bioorganic and medicinal chemistry of antimicrobial compounds. Three main projects are: a) Synthesis of molecular tools for study of bacterial stringent response and bacterial tox/antitox systems, b) Synthesis of azanucleotide inhibitors of nucleotide selvage pathway as potential antimalarials and antituberculotics, and c) synthesis, evaluation, and application of membrane targeting antibacterial compounds lipophosphonoxins. https://rejman.group.uochb.cz/en

The Rosenberg group is interested in the synthesis and biochemical and biological evaluation of structurally diverse sugar-modified nucleoside phosphonic acids (NPA) and chimeric oligonucleotides (ON) composed of both natural and phosphonate nucleotide units. Among enzyme-stable NPAs, a potent multisubstrate-like inhibitors of *nucleotide and nucleoside salvage pathway* enzymes were discovered. Several types of chimeric phosphonate ONs were found as promising (i) antisense compounds working by RNase H mechanism and by steric block and (ii) agonists of TLR 9 in case of CpG motif containing ONs. The Group is also interested in new methods of phosphonate ONs synthesis. https://rosenberg.group.uochb.cz/en

Synthetic organic, materials and nano chemistry

The team **Synthetic organic, materials and nano chemistry** consists of four independent research groups focusing on the development of original synthetic methodologies for use in various fields of organic, materials and nano chemistry. The main attention is paid to the total synthesis of natural and non-natural products, heteroanalogues of graphene, chemistry of main group elements (B, F, S, Si, Sn), non-trivial π-electron architectures, catalysis, photo- and radical chemistry. Singlet fission, molecular rotors, nanoparticles for medicinal applications, chiroptics and charge transport are also investigated. Basic research dominates the activities of all senior research groups in the cluster: Dr. Petr Beier, Dr. Petr Cígler, Dr. Ullrich Jahn, Prof. Josef Michl (Distinguished Emeritus) and Dr. Ivo Starý.

The Beier group is active in the development of new, selective and convenient synthetic reagents and methods towards novel organic molecules, which can find applications in crop protection, drug design and in materials. We study new reactions and their mechanisms. We are focusing on organic chemistry of the main group elements such as fluorine, phosphorus, silicon, sulfur and iodine. We have been active in methodology development of fluorinated phosphonates, pentafluorosulfanyl containing compounds, fluoroalkylations, transfer of tetrafluoroethyl and tetrafluoroethylene fragments, bioconjugations, and the chemistry of fluorinated azides, heterocycles, enamines and enamides. More info: https://beier.group.uochb.cz/en.

The Cígler group works in synthetic chemistry of nanoparticles with focus on bioimaging and sensing. This topic is solved using cross-disciplinary approach involving organic and inorganic synthesis, protein expression, spectroscopy, and physics. Cigler group pioneered design and

preparation of nanosensors providing selective and ultrasensitive detection of chemical processes using so-called quantum sensing. The sensors are based on diamond nanocrystals and enable localized optical detection of ~10^{-22} mol of molecules. The group developed also highly biocompatible nanoparticle interfaces for applications in biomedicine. More info: http://nano.petrcigler.cz/

The Jahn group. The major focus of the Jahn group lays on synthetic organic natural product chemistry. Major target classes of the group are alkaloids, steroids, terpenoids, lignans and autoxidatively formed lipid metabolites. Major aims are the establishment of absolute and relative configuration of isolated natural products, the provision of natural products and of analogs for biological study, access to molecular probes to elucidate mechanisms of action at biological targets and synthetic approaches to non-natural amino acids for incorporation into peptides or foldamers. These target-oriented approaches are supported by strong methodology development, especially in radical chemistry, gold catalysis, the development of tandem reactions, or photochemical amination reactions, to name a few. More info: https://jahn.group.uochb.cz/en.

The Michl group is focused on field of materials chemistry in several areas: Singlet fission, gold surface alkylation, molecular rotors and boron and fluorine chemistry. The singlet fission program deals with theoretical and practical aspects, which might open door to practical use of this phenomenon – hybrid solar panel manufacturing. In gold surface alkylation project, we have been following the mechanism and characterization of alkyl monolayer formation by contact of alkylstannane solution with gold surface. An exploration of the properties of assemblies of dipolar molecular rotors carried by surfaces or thin layers found out much about their behavior (including evidence of ferroelectric interactions). In boron and fluorine chemistry efforts have been centered on the exploratory chemistry of closo-carborane anions derived by substitution from $CB_{11}H_{12}(-)$ and the related radicals, $CB_{11}H_{12}(\bullet)$. Many of them showed extraordinarily high oxidation potentials (one more than 4V above Fc/Fc^{+}). More info: https://michl.group.uochb.cz/en.

The Starý group focuses on non-trivial π-electron architectures that are attractive for applications in chemistry and physics. Particular attention is paid to the synthesis of helically chiral aromatics (helicenes), which are enantiopure and properly functionalized. The group systematically investigates their (chir)optical properties, self-assembly in crystals or at interfaces, charge/spin transport properties and on-surface reactivity at nanoscale. There is also great interest in the general synthetic methodology development and enantioselective catalysis. The ultimate goal of multidisciplinary efforts is the development of smart molecular devices. More info: https://stary.group.uochb.cz/en.

Biochemistry and Molecular Biology

The **Molecular interactions in biomedicine team** (Biology I, BIO I) consists of 3 senior groups: J. Konvalinka (JK), I. Pichová (IP), M. Mareš (MM), 2 junior groups (evaluated and transformed into senior groups in 2019: E. Bouřa (EB), K. Stříšovský (KS)), 2 research service groups: (H. Mertlíková (HM), J. Weber (JW)), and targeted research group (G. Birkuš (GB)). Majority of these groups (JK, IP, MM. EB, KS, HM) collaborated already within the Centre of Molecular Interactions in Biomedicine (CZ.2.16/3.1.00/24016CZ.2.16), which was established in 2010 and was supported by European structural funds in years 2010-2012 and later from National programme for sustainability (NPU, LO 1302) in years 2014-2019. Groups of JW and GB joined this team later and closely collaborated with the whole cluster. Research of Bio I team was oriented to molecular and chemical biology, medicinal chemistry, structure biology, pharmacokinetics and was focused on viral, bacterial, and parasitic pathogens, tumor diseases, and development of novel approaches and medications of different diseases. All

groups performed independent research but shared know how, common equipment and facilities. Focus of individual groups is following:

The Konvalinka group

The main focus of the Konvalinka group is identification, validation and characterization of traditional or novel therapeutic targets for the diagnostics and treatment of viral diseases and cancer. We combine several approaches to achieve these goals including synthetic chemistry, chemical biology, structural biology and biochemistry. Our models involve HIV, HBV, Influenza, Zika, Dengue and other human viruses, prostate cancer, human glioblastoma and other severe pathological conditions. We use wide variety of methodologies spanning from organic synthesis, molecular modelling and medicinal chemistry, recombinant protein production in various organisms, enzymology and X-ray and NMR structure analysis to mammalian cell cultures, xenografts of human tumors and transgenic mice. Recently, our group developed novel chemical biology tools based on the conjugates of specific ligands with either biocompatible polymers or DNA oligonucleotides (iBodies, DIANA). They could be used for identification, isolation, visualization and quantification of a variety of protein targets and for high-throughput testing of their inhibitors and ligands. Using these tools, we search for novel inhibitors of known target enzymes (tumor and viral enzymes) as well as for new therapeutic targets. More information available at https://konvalinka.group.uochb.cz/en

The Pichová group research is primarily focused on investigation of different aspects of life cycles of selected human pathogens: retroviruses, *Hepatitis B virus*, *Mycobacterium tuberculosis* and their interaction with host cells. Part of the reseach is targetted in to chemical biology and biosynthesis of modified fatty acids. The research is interdisciplinary, combines molecular biology experiments such as gene silencing, qRT PCR analyses of gene expression, next generation sequencing, protein engineering, *in vitro* characterization of proteins, structural studies with computation analyses. This interdisciplinary approach is based on collaboration with number of researchers from different fields. More information available at https://pichova.group.uochb.cz/en

The Mareš group is focused on cathepsin proteases and proteolytic systems controlled by these enzymes. They play a critical role in pathologies such as cancer, osteoporosis, neurodegenerative, and immune diseases, and are essential for viability or virulence of important human parasites. The research aims at identifying mechanisms of function and regulation of cathepsins, and developing of cathepsin inhibitors as chemotherapeutics. More information available at https://mares.group.uochb.cz/en

The Bouřa group works in the field of structural and molecular biology of membrane associated proteins such as lipid transport proteins, lipid kinases and membrane associated viral enzymes. All the single-stranded plus RNA (+RNA) viruses replicate on the surface of host membranes. Recently the focus of the boura group were the RNA-dependent RNA polymerases (RdRps) and methyltransferases (MTases) from the viral families Flaviviridae and Coronaviridae. The group is using X-ray crystallography, biochemistry and enzymology to characterize these enzymes in vitro and to prepare inhibitors that could serve as potential antivirals. More information available at https://boura.group.uochb.cz/en

The Stříšovský group studies intramembrane proteolysis and membrane protein biogenesis and quality control at the interface of cell biology, structural biology and chemical biology. The Strisovsky group has explained substrate specificity of rhomboid proteases structurally, and designed first high-affinity specific and biocompatible inhibitors of these enzymes. The group uses proteomics, cell biology and mouse models to understand the biological roles of rhomboid proteases in particular in growth factor signalling, lung biology and immunity. More information available at https://strisovsky.group.uochb.cz/en

The Mertlíková group (Biochemical Pharmacology) represents the so-called research-service organization unit which primarily aims to support other IOCB teams in their research activities by offering routine and advanced services (flow cytometry, cell culture, animal facility and ADME/Tox). These include for example assay development, operation and maintenance of the state-of-art instruments, user training and consultations. The group is focused on drug discovery projects and its research is almost exclusively elaborated on the grounds of screening campaigns findings in order to promote new interesting small molecules from hit identification phase further. This often includes target validation or looking for the mechanism of action. The expertise of the group is particularly strong in the field of anti-tumor and anti-inflammatory drugs but it is rather flexible and capable of reacting on the actual needs. More information available at https://pharmacology.group.uochb.cz/en

The Weber research-service group works on different virus-related projects with focus on HIV, HBV and virucidal nanoparticles. Group provides antiviral compound screening and service for other IOCB groups in projects involving viruses. Their research projects cover areas such as HIV inhibition, reactivation, latency and HIV fitness, HBV core protein interactions with host cell proteins and search of nanomaterials with antiviral and virucidal activity. More information available at https://virology.group.uochb.cz/en

The Birkus targeted research group performs drug discovery with a focus on unmet medical needs. The team is cross functional and has capability of synthesis, crystallography, computational modelling and *in vitro* and *in vivo* biological profiling of prepared compounds. More information available at https://birkus.group.uochb.cz/en

Chemical biology for life and diseases

The team **Chemical biology for life and diseases** (Biology II, BIO II) consists of several research groups of different types and status: Senior Research Groups of Dr. P. Maloy Řezáčová, Dr. J. Jiráček and Dr. L. Maletínská, Junior Groups of Dr. H. Cahová, Dr. E. Curtis, Dr. R. Hanus, Dr. Z. Kečkéšová and Dr. N. Weiss and Research Service Group of Dr. J. Vondrášek. The specific focuses of individual groups are described below in details.

This team studies biological interactions implicated in various pathologies. We use cutting-edge methods of protein structural biology and bioinformatics, peptide hormone biochemistry and physiology, RNA biology, tumour cell biology and insect chemical ecology to understand biological processes and develop new therapeutic interventions and potential medicines. We aim to achieve excellence in basic research while also keeping practical applications in mind.

The **Maloy Řezáčová group** is focused on identification and validation of key biological interactions implicated in human pathologies that could be targeted in therapeutic intervention. This requires detailed understanding of molecular mechanisms of various biological processes using the means of integrative structural biology. In particular, we combine our expertise protein biochemistry, X-ray crystallography, biomolecular nuclear magnetic resonance spectroscopy, single-particle cryo-electron microscopy with cell biology and biophysics. We study bacterial and eukaryotic transcription regulation as well as various enzymatic networks linked to cancer development viral infection.
More information available at https://rezacova.group.uochb.cz/

The **Jiráček group** is interested in all aspects of insulin and insulin-like growth factors 1 and 2 (IGF-1/2) physiology. These important hormones share similar 3D structures and cell membrane receptors. Insulin and IGFs cross-bind to these receptors with different affinities and trigger distinct but overlapping physiological effects; predominantly metabolic for insulin and predominantly mitogenic for IGFs. Insulin/IGF system plays a major role in the regulation of metabolism, growth, development and lifespan. In addition, it has a role in the development

of cancer, diabetes, growth-related and neurological diseases. Our general goal is understanding of the structural basis for the different cellular responses, metabolic and mitogenic, generated by insulin and IGFs. We synthesize analogues and mimetics of insulin and IGFs to study their interactions with cognate receptors and to develop new drugs for treatment of hormone-related disorders. More information available at https://jiracek.group.uochb.cz/

The **Maletínská group** is focused on peptides involved in the regulation of food intake and processes related to obesity, diabetes and neurodegeneration. It combines biochemical, pharmacological and physiological methods and aims at clarifying the relationships among peptides involved in development of obesity in order to design possible therapeutics. Recently discovered anorexigenic neuropeptides, e.g. prolactin-releasing peptide (PrRP) and cocaine and amphetamine-regulated transcript (CART) peptide, represent new trends in development of anti-obesity agents. They target directly brain areas regulating food intake, but generally do not cross the blood-brain barrier if administered peripherally. We designed stable lipidized analogs of PrRP capable to act centrally, with prolonged half-lives and anti-obesity and antidiabetic effects after peripheral administration in mice and rats with diet induced obesity and insulin resistance. Type 2 diabetes and obesity were shown to be a risk factors for Alzheimer's disease (AD), thus compounds with glucose lowering and/or anorexigenic properties were proposed to be neuroprotective. We demonstrated that PrRP is a potential neuroprotective tool improving spatial memory and attenuated two hallmarks of AD, Tau hyper-phosphorylation and β-amyloid plaques in mice with neurodegeneration.
More information available at https://maletinska.group.uochb.cz/

The **Cahová group** was established in 2016 and focuses on RNA modifications. During years 2016-2019, the group studied chemical RNA modifications in two model systems: viruses and bacteria. More information available at https://cahova.group.uochb.cz/

The **Curtis group** studies the function potential of nucleic acids. Many projects in the group use a powerful technique of artificial evolution called *in vitro* selection, which can be used to isolate rare DNA and RNA sequences with enzymatic activity from large random sequence pools. We are particularly interested in developing new signalling components that can be used in allosterically regulated DNA and RNA sensors. We also study the functions of G-quadruplexes using a variety of biochemical and structural approaches.
More information available at https://curtis.group.uochb.cz

The **Hanus group** studies the biology and chemistry of social insects. The group combines the techniques of analytical chemistry, gas-phase metabolomics, sensory physiology, biochemistry and molecular genetics to address diverse questions, which can be classified into three main fields: (i) chemical ecology of social insects (termites, bumblebees), namely the diversity of exocrine chemicals (pheromones, defensive compounds), their biosynthesis and biological significance; (ii) genetic mechanisms of reproduction of termite colonies; (iii) physiological mechanisms controlling insect development, caste differentiation and longevity of social insects. More information available at https://hanus.group.uochb.cz

The **Weiss group** is interested in voltage-gated-calcium channels that are the primary mediators of the depolarization-induced calcium entry into neurons that initiates many cellular events. While calcium channels are of critical importance for neuronal function, it is also apparent that inappropriate expression or dysfunction gives rise to a variety of neurological disorders investigates the intrinsic gating processes, as well as cell signalling pathways that control channel activity and trafficking to and from the plasma membrane, and how these regulations are compromised in disease states or by genetic mutations. Specifically, we focus on the subfamily of T-type calcium channels that are implicated in several neurological conditions, including neuropathic pain and epilepsy.
More information available at https://weiss.group.uochb.cz

The **Kečkéšová group** was established in 2018 as a junior group and focuses on identification and characterization of novel tumour suppressor pathways in cancer.
More information available at https://keckesova.group.uochb.cz

The **Vondrášek group** primarily focuses on biomolecules, their sequences, evolution, structures, architectures, interactions and complexes. Special attention is paid to evolutional pathways in which a function emerged and was further optimized. Group is not limited to work in sequence-structure-function paradigm but also includes studies of Intrinsically Disordered Proteins. Vondrášek's group use combinations of molecular modelling, molecular simulations, computational chemistry, bioinformatics analysis and mathematical statistics methods and run experimental laboratory dedicated to production of designed proteins and their characterization. The group mission is to establish a robust methodological background suitable to provide solution of various structural biology and life science related problems.
More information available at https://bioinformatics.group.uochb.cz/

Molecular modelling and spectroscopy in chemistry and biology

The **Molecular modeling and spectroscopy in chemistry and biology** team (Physical Chemistry I, PHYS I) comprises *five groups*, each led by a single principle investigator (PI), namely Prof. Pavel Hobza, Dr. Zdeněk Havlas, Prof. Pavel Jungwirth, Doc. Lubomír Rulíšek, and Dr. Josef Lazar. They closely collaborate, both within the Physical Chemistry I team (e.g. joint theoretical chemistry seminars, several joint publications, …) as well as with their experimental colleagues from other IOCB teams. Their expertise and research cover more or less full spectrum of problems, methods, and approaches in theoretical and quantum chemistry and molecular modelling; and they also include advanced spectroscopic methods (Josef Lazar). Below, expertise and main research focus is mentioned separately for each group:

The Hobza group specializes in reliable computational description of noncovalent interactions, using and developing various quantum chemical methods, ranging from highly accurate CCSD(T)/CBS approaches to semiempirical ones, such as PM6-D3H4X. The topics cover important areas of chemistry, such as *in silico drug* or material design. The cornerstone of the research are databases of noncovalently interacting small model systems, which can be treated with the most accurate methods. These serve for the development of more approximate ones, which in turn can be used for systems of thousands of atoms. More info: https://hobza.group.uochb.cz/

The Havlas group is focused on the theoretical studies of reactions and properties of organic and bioinorganic compounds with complex electronic structure, such as biradicals and transition metal compounds. This includes a search for new chromophores which might represent suitable candidates for singlet fission process, a promising alternative for improving the efficiency of organic solar cells. Scientists in the group are also interested in spin-dependent relativistic effects (spin-orbit and spin-spin couplings, electronic energy shift due to parity violation), as well as in advanced spectroscopies (EPR, CD, MCD). The research is based mostly on modern multi-reference electronic structure methods. Apart from the high-level calculations, group is engaged also in the methodology development and scientific programming. More info: https://havlas.group.uochb.cz/

The Jungwirth group has been aiming primarily at gaining molecular level understanding of biological processes involving ions using computer simulations in close contact with spectroscopic experiments. Using molecular dynamics simulations and quantum chemical methods the group has been attempting to establish the mechanisms of ion-protein interactions responsible for the salting out (Hofmeister) series and beyond. Applications of the group's research span from influencing protein aggregation, precipitation or denaturation and

controlling enzymatic activity to establishing properties of phospholipid bilayers in the presence of ions. One of the key aims within the latter subject has been to establish molecular principles governing the action of calcium ions involved in membrane fusion and cationic cell penetrating peptides important, e.g., for novel ways of drug delivery to cells. Related research activities of Jungwirth's group concern electron solvation pertinent to radiation chemistry and the Birch reduction process. In free time, the group entertains itself by performing "balcony experiments" involving, for example, explosions of alkali metals in water, which also allows to connect to general public and popularize science. More info: http://jungwirth.uochb.cas.cz/

The Rulíšek group focuses on understanding catalytic action of metalloproteins by employing modern quantum mechanical methods for realistic systems, accurate solvation models and QM/MM-like coupling schemes along with bioinformatics or structural search engines. Ultimate goal is *ab initio* design of small catalytic metallopeptides and highly specific metal chelators. Other research topics in the group include development of quantum and molecular mechanical (QM/MM) methods, organic reactivity, computational homogeneous catalysis, protein-ligand interactions, computational electrochemistry, theoretical spectroscopy, relativistic quantum chemistry, and design of novel fullerenes that can act as molecular switches, transistors, and memristors. Our recent contributions to general chemical knowledge include theoretical and experimental proof of hydrogen bonding to gold or to understanding of heavy-atom effects on NMR chemical shifts across the periodic table. https://rulisek.group.uochb.cz/

The Lazar group works on developing techniques of advanced optical microscopy and applying them to studies of molecular processes taking place in living cells and organisms. The main focus of the group is the technique of two-photon polarization microscopy (2PPM), developed by the group. The group uses this technique to investigate molecular events involved in G-protein signalling, insulin and IGF signalling, and other processes. In order to expand the uses of the technique, the group develops genetically encoded molecular probes for 2PPM, as well as investigates optical properties of fluorescent proteins that form the basis of these probes. The team has a personal and activity overlap with the Laboratory of Advanced Optical Microscopy of the Institute of Microbiology CAS in Nove Hrady.

Spectral analytical methods and separations

The team **Spectral analytical methods and separations** (Physical chemistry II, PHYS II) is focused on the development of analytical instrumental spectroscopic and separation methods and their application for the study of structure, properties, and functions of various compounds, from small molecules to biomacromolecules. The research is focused on the fundamental theory, methodology, instrumentation, and applications of NMR, MS, chiroptical spectroscopy, and electroseparation methods. The team consists of the Senior Research Group of Prof. P. Bouř and three Research-Service Groups of Assoc. Prof. J. Cvačka, Dr. V. Kašička, and Dr. D. Šaman. In addition to their research projects, all groups provide wide support and collaboration to other IOCB groups in using the advanced instrumental techniques.

The Bouř group, Biomolecular Spectroscopy**,** is dedicated to the development of theoretical and experimental methods needed for biomolecular spectroscopy and imaging. Within 2015-2020 the group developed, for example, computational algorithms and program for magnetic circular dichroism, vibrational optical activity of complex biomolecular systems, and corrections beyond the Born-Oppenheimer approach. The group also described new experimental procedures, potentially useful for analytical chemistry, such as resonance Raman optical activity of gases and induced circularly polarized luminescence of lanthanide probes. More info at https://bour.group.uochb.cz/en

The Cvačka group, Mass Spectrometry, analyses small compounds such as products of organic synthesis or natural compounds, as well as larger molecules like proteins or

oligonucleotides. In addition to qualitative and quantitative analysis of the samples, mass spectrometry is used to visualize the spatial distribution of various compounds in tissue sections. The group also implements procedures for characterizing the three-dimensional structure of proteins and their interactions. Research projects comprise the development of the method, instrumentation, and applications of mass spectrometry in biosciences, particularly for structural analysis lipids and proteins. More info at https://ms.group.uochb.cz/en

The Kašička group, Electromigration Methods, deals with the research and development of theory, methodology, instrumentation, and application of high-performance capillary electromigration (CE) methods. Methodology developments include all major CE techniques: zone electrophoresis, affinity electrophoresis, isotachophoresis, isoelectric focusing, electrokinetic chromatography, and electrochromatography. In the area of instrumentation, new devices for one- and two-dimensional CE methods with a multidimensional detection system are being developed. The developed instrumentation and methods are applied for the fast and high-efficient separation, high-sensitive qualitative and quantitative analysis, and physicochemical and biochemical characterization of biomolecules and functional organic molecules. Besides, the cocktail effect of triazole fungicides on essential enzymes in the human body is studied. More info at https://electromigration.group.uochb.cz/en

The Šaman group, NMR Spectroscopy, operates eight nuclear magnetic resonance (NMR) spectrometers and an electron paramagnetic resonance (EPR) spectrometer. It provides NMR and EPR service to all interested researchers from the IOCB. An important activity of the group is managing self-service measurement on two 400 MHz spectrometers for more than 190 users including training of new users, troubleshooting, and setting of tailored NMR experiments. Many research projects are initiated by other IOCB groups and most often involve structural analysis of newly prepared or isolated compounds, and determination of reaction mechanisms and reaction kinetics. Such “intramural” collaborative research led to ca 190 peer-review publications in 2015–2019. The research also involves fundamental aspects of experimental NMR/EPR spectroscopy of solutions and solids, molecular modelling, and theoretical calculations of spectroscopic parameters and molecular properties. More info at https://nmr.group.uochb.cz/en

Research activity and characterisation of the main scientific results

The Hobza group has been involved primarily in the following four research activities: (i) benchmark databases of noncovalently interacting model systems, and their use in development of approximate QM methods; (ii) *in silico* drug design; (iii) computational material design; (iv) modelling of excited states of graphene quantum dots.

Ad (i), benchmark databases: Quantum mechanics is in principle able to give ultimate answers about a chemical structure. Unfortunately, the exact solutions of the Schrödinger equation are reserved for a few smallest systems only and the QM methods applicable to larger molecules only approximate. To test the accuracy of these methods, and to develop new ones, reliable reference data are needed. Because of limited experimental evidence on non-covalent interactions in isolated systems, reliable calculations (using the "gold standard" CCSD(T)/CBS methodology) are widely used as a benchmark. We are one of few groups who analyzed the accuracy of this benchmark itself by the means of even more advanced calculations.[PH1] We are active in developing comprehensive databases of benchmark data for non-covalent interactions, and our databases became the most widely used benchmark in the field. Our earlier work had been summarized in a detailed review.[PH2] Recently, we have focused on developing a next generation of larger databases covering wider chemical space.[PH2b] We have created the Non-Covalent Interaction Atlas repository (www.nciatlas.org) where these data are published. Apart from the benchmark calculations, we also study the physical nature of non-covalent interactions in interesting systems.[PH2c]

We use our benchmark data to develop new approximate methods that maximize the accuracy that can be achieved at a reduced computational cost, ranging from DFT [PH2d] to semiempirical QM methods.[PH2e]

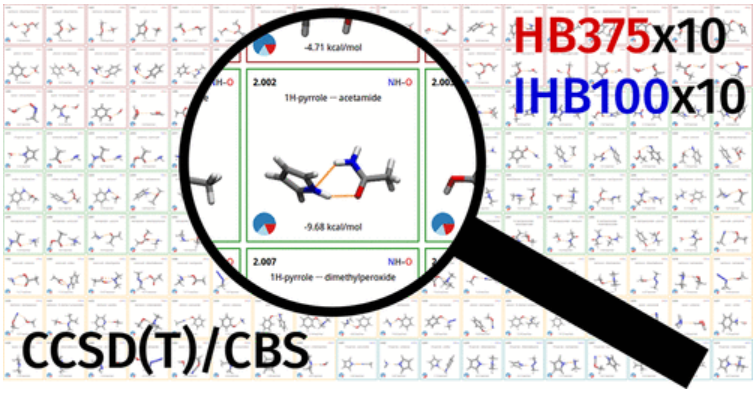


Figure 1.1: Non-Covalent Interaction Atlas database web repository of benchmark CCSD(T)/CBS data.

Ad (ii), in silico drug design: We have taken a systematic approach to design a modular general-purpose semiempirical QM (SQM)-based scoring function [PH3]. We have adopted the MM-PB/GBSA-like master equation in which the interaction- and solvation-related terms (i.e. all except entropy) are evaluated using SQM (and for reference also QM) methods and COSMO/SMD, respectively. The modularity means that different levels of theory can be combined for interaction- and solvation-related terms because they arise due to different physicochemical phenomena. The score which approximates the binding free energy is computed on the protein-ligand complex optimized in aqueous environment. The individual terms describe the gas-phase interaction energy, the change of solvation free energy upon complex formation, the change of conformational 'free' energy of the protein and ligand in aqueous environment and the change of entropy upon ligand binding. The reliable estimation

of the first term, which is the dominant and only favorable in the equation, is crucial. The unfavourable solvation/desolvation represents the second largest term, especially for charged ligands or protein binding sites and its estimation is difficult and connected with larger uncertainties. Both terms thus partially compensate for each other and the final score is considerably smaller than their absolute values. The other terms are about one or two orders of magnitude smaller than the first two terms. The above mentioned SQM-based scoring function has been successfully applied using different levels of theory for ranking (scoring power) of inhibitors of various kinases, proteases, aldo-keto reductases [PH4] and also of serine racemase.[5] Presence of explicit water molecules in protein binding sites [PH5] or unusual noncovalent interactions, such as halogen bonding [PH6,7] does not pose any problems to the SQM-based scoring function, in contrast to the standardly used ones.

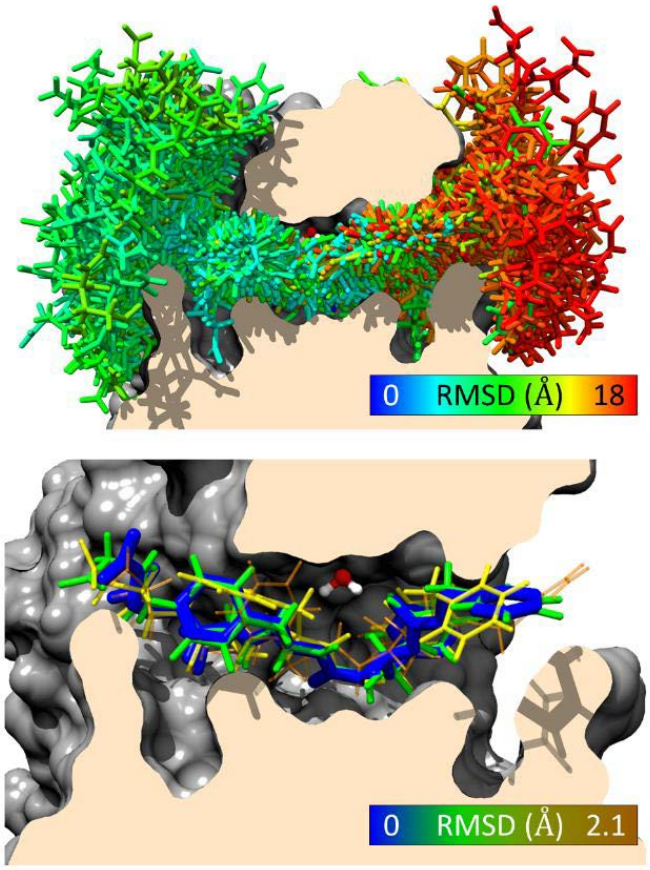


Figure 1.2 The SQM/COSMO scoring function selects unequivocally from thousands of docking poses (top) the native one (bottom, in blue sticks) in the binding site of HIV-1 protease.

Ad (iii), computational material design: First-principle quantum mechanical studies have been performed aiming to control the structural and electronic properties of nano-materials (e.g. graphene and other carbon allotropes) and organo-metallic systems by covalent and non-covalent functionalization. The calculations have been performed to obtain detailed insight into interactions upon functionalization at the atomic scale. Description of non-covalent interactions, in particular, represents challenging problem and, thus, its treatment at the high computational levels is highly desirable [PH8,9,10]. The computational approach based on the DFT method augmented with a proper treatment of dispersion forces proved to be provide a reliable description of these interactions when compared with experiment [PH9,10]. The calculations performed in Hobza group concentrate also on description of electronic properties of carbon allotropes and their control by heteroatom doping [PH11]. Combined experimental and computational studies have been performed on Iron(II) phthalocyanine (FePc), an

important member of the phthalocyanines family with potential applications in the fields of electrocatalysis, magnetic switching, electrochemical sensing, and phototheranostics. Despite the importance of this systems the nature of the ground state of FePc and other Fe-porphyrine systems is still debating. The challenge of its determination comes from almost degeneracy of the spin states due to a large number of energetically close-lying molecular orbitals, which requires the use of multireference description, and easy spin-state switch in different environments. Using Mossbauer spectroscopy and Superconducting Quantum Interference Device (SQUID) magnetic measurements performed in frozen monochlorbenzene, which guarantees isolated character of FePc, and multi-reference complete active space second-order perturbation theory (CASPT2) and density matrix renormalization group (DMRG) methods assign quintet as the FePc ground-state in the gas-phase [PH12]. The control of the FePc spin state has been demonstrated experimentally by its sensing by atomic force microscopy with CO-functionalized tip when located on the pristine and on N-doped graphene. The calculations were able to interpret the experiment by changing the spin state of FePc driven by weak intermixing between orbitals with z-component of N-dopant (p(z) of N-dopant) and molecule (d(xz), d(yz), d(z)(2)) with subsequent reordering of the Fe d-orbitals [PH13]

Ad (iv): Graphene quantum dots (CD) represent promising material with applications in cell labeling, optical imaging, LED diodes, and optoelectronic and biomedical technologies. The experimental studies, however, show a highly complex behaviour of these systems which makes it difficult to fully explore their photoluminescence behaviour. Reliable calculations of such systems are thus highly desirable. Several studies [PH14,15,16] have been performed in the Hobza group on the model systems of quantum dots in which the popular DFT method is tested with respect to available experiment and/or wave-function-based methods, including a multi-reference approach which can help to select a proper methodology to be used in the calculations of more larger, more realistic models.

Selected key publications (Hobza group): (PH1) Rezac *et al*.: Extensions and applications of the A24 data set of accurate interaction energies. *Phys. Chem. Chem Phys.* **2015**, 17, 19268-19277; (PH2) Rezac *et al.*: Benchmark Calculations of Interaction Energies in Noncovalent Complexes and Their Applications. *Chem. Rev.* **2016**, 116, 5038-5071; (PH2b) Rezac *et al.*: Non-Covalent Interactions Atlas Benchmark Data Sets: Hydrogen Bonding. *J. Chem. Theory Comput.* **2020**, 16, 4, 2355–2368; (PH2c) Rezac *et al.*: On the role of charge transfer in halogen bonding. *Phys. Chem. Chem. Phys*. **2017**,19, 791-803; (PH2d) Hostas *et al.*: Accurate DFT-D3 Calculations in a Small Basis Set. *J. Chem. Theory Comput.* **2017**, 13, 8, 3575–3585; (PH2e) Rezac *et al.*: Empirical Self-Consistent Correction for the Description of Hydrogen Bonds in DFTB3. *J. Chem. Theory Comput*. **2017**, 13, 10, 4804–4817; (PH3) Pecina *et al.*: The SQM/COSMO filter: reliable native pose identification based on the quantum-mechanical description of protein-ligand interactions and implicit COSMO solvation. *Chem. Comm*. **2016**, 52, 16, 3312-3315; (PH4) Fanfrlik *et al.*: The Effect of Halogen-to-Hydrogen Bond Substitution on Human Aldose Reductase Inhibition. *ACS Chem. Biol.* **2015**, 10, 7, 1637-1642; (PH5) Vorlova *et al.*: Malonate-based inhibitors of mammalian serine racemase: Kinetic characterization and structure-based computational study. *Eur. J. Med. Chem*. **2015**, 89, 189-197; (PH6) Kolar *et al.*: Computer Modeling of Halogen Bonds and Other sigma-Hole Interactions. *Chem. Rev* **2016**, 116, 9, SI, 5155-5187; (PH7) Sedlak *et al.*: Polar Flattening and the Strength of Halogen Bonding. *J. Chem. Theory Comput.* **2015**, 11, 10, 4727-4732; (PH8) Kalicky *et al.:* Adsorption of Organic Molecules to van der Waals Materials: Comparison of Fluorographene and Fluorographite with Graphene and Graphite. *J. Chem. Theory Comput.* **2017**, 13, 3, 1328–1340; (PH9) Sedlak *et al.*: The role of the sigma-holes in stability of non-bonded chalcogenide ... benzene interactions: the ground and excited states. *Phys. Chem. Chem Phys.* **2018**, 20, 299-306; (PH10) Hostas *et al.*: A Nexus between Theory and Experiment: Non-Empirical Quantum Mechanical Computational Methodology Applied to Cucurbit[n]urilGuest Binding Interaction. *Chem. Eur. J.* **2016**, 22, 17226-17238; (PH11) Sarmah *et al.*: Sequential BN-doping induced tuning of electronic properties in zigzag-edged

graphene nanoribbons: a computational approach. *RSC Adv.* **2018**, 8, 10964-10974; (PH12) Nachtigallova *et al.*: An Isolated Molecule of Iron(II) Phthalocyanin Exhibits Quintet Ground-State: A Nexus between Theory and Experiment. *Chem. Eur. J* **2018**, 24, 13413-13417; (PH13) da la Tore *et al.*: Non-covalent control of spin-state in metal-organic complex by positioning on N-doped graphene.*Nat. Comm.* **2018**, 9, Article Number: 2831; (PH14) Bettanin *et al.*: Singlet L-a and L-b Bands for N-Acenes (N=2-7): A CASSCF/CASPT2 Study. *J. Chem. Theory Comput.* **2017**, 13 4297-4306; (PH15) Shi *et al.*: Excited states and excitonic interactions in prototypic polycyclic aromatic hydrocarbon dimers as models for graphitic interactions in carbon dots. *Phys. Chem. Chem Phys.* **2019**, 21, 9077-9088; (PH16) Shi *et al.*: High-level theoretical benchmark investigations of the UV-vis absorption spectra of paradigmatic polycyclic aromatic hydrocarbons as models for graphene quantum dots. J. Chem. Phys. **2019**,150, Article Number: 124302.

The Havlas group has conducted theoretical studies for several molecules, which can be considered either potential candidates for singlet fission process or potential photosensitizers for photodynamic therapy. We have provided a rationale for the use of captodative biradicaloids for singlet fission, i.e., biradicals stabilized by direct interaction between their radical centers, which carry both an acceptor and a donor group, such as benzoquinone derivatives, some of which show promising relative energies of states involved in singlet fission [ZH1]. We have described a procedure for unbiased identification of chromophore pair geometry choices that locally maximize the rate of conversion of a singlet exciton into a triplet pair necessary for singlet fission [ZH2]. The low-lying states of difluoroborondipyrromethene (BODIPY) monomer and dimer were revisited, correcting the previously found artifact states [ZH3], and fundamental photophysical properties and interactions with biological environments of halogenated BODIPY derivatives, which may serve as sensitizers in photodynamic therapy, were probed by spin-forbidden nonadiabatic molecular dynamics [ZH4]. We have contributed to the development of modern multireference computational methods [ZH5], as well as to the development of theoretical methodology for magnetic circular dichroism spectroscopy and its applications to polycyclic aromatic hydrocarbons and other molecules [ZH6].

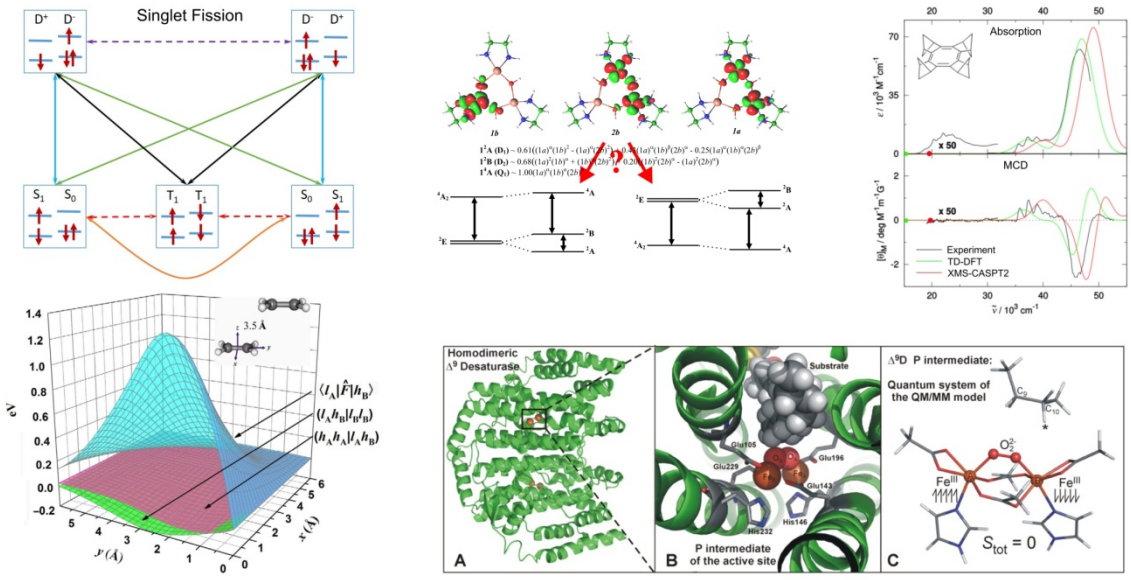


Figure 1.3: *Representative examples of Havlas group projects*: Singlet fission mechanism and ethylene pair (left), mechanism of multicopper oxidase ground-state spin-orbit coupling (top middle); structure of the non-heme binuclear iron site in □9 desaturase (botton center and

right), absorption and magnetic circular dichroism spectra of annulene computed by multi-reference wave function methods.

Selected key publications (Havlas group): (ZH1) Wen *et al.*: Captodatively Stabilized Biradicaloids as Chromophores for Singlet Fission. *J. Am. Chem. Soc.* **2015**, *137*, 165-172. (ZH2) Zaykov *et al.*: Singlet Fission Rate: Optimized Packing of a Molecular Pair. Ethylene as a Model. *J. Am. Chem. Soc.* **2019**, *141*, 17729-17743. (ZH3) Wen *et al.*: An MS-CASPT2 Calculation of the Excited Electronic States of an Axial Difluoroborondipyrromethene (BODIPY) Dimer. *J. Chem. Theory Comput.* **2018**, *14*, 4291-4297. (ZH4) Pederzoli *et al.*: Photophysics of BODIPY-Based Photosensitizer for Photodynamic Therapy: Surface Hopping and Classical Molecular Dynamics. *J. Chem. Theory Comput.* **2019**, *15*, 5046-5057. (ZH5) Yanai *et al.*: Density matrix renormalization group for *ab initio* Calculations and associated dynamic correlation methods: A review of theory and applications. *Int. J. Quantum Chem.* **2015**, *115*, 283-299. (ZH6) Kaminský, J. *et al.*: Vibrational Structure in Magnetic Circular Dichroism Spectra of Polycyclic Aromatic Hydrocarbons. *J. Phys. Chem. A* **2017**, *121*, 9064-9073.

The Jungwirth group has been involved primarily in the following three research activities: (i) Interactions of ions and cationic peptides with phospholipid membranes. (ii) Structure, dynamics, and reactivity of solvated electrons. (iii) Development of force fields for molecular simulations with implicit inclusion of electronic polarization.

Ad (i), Interactions of ions and cationic peptides with phospholipid membranes: Jungwirth's group has been systematically investigating the interactions of biologically relevant ions (calcium in particular) and cationic cell penetrating peptides with membranes of varying phospholipid and cholesterol compositions. One of the principal goals has been to establish a mechanistic connection between passive cell penetration and membrane fusion, which controls vital cellular processes ranging from shaping cell morphology to neuronal signalling. While many of these processes are tightly regulated, e.g., by the SNARE protein complex in the case of fusion of synaptic vesicles with the neuronal cell membrane, spontaneous fusion and fission is frequently observed in cellular and model membranes. For example, even for the strongly regulated neuronal signalling, unregulated vesicle fusion also occurs spontaneously, likely due to natural fluctuations of local calcium concentration.

Jungwirth's group has recently shown that calcium binding to model membranes depends strongly on curvature and lipid composition, becoming stronger in positively curved regions and/or in the presence of negatively charged phospholipids. For the first time, it has been shown using atomistic simulations that calcium can induce membrane fusion in

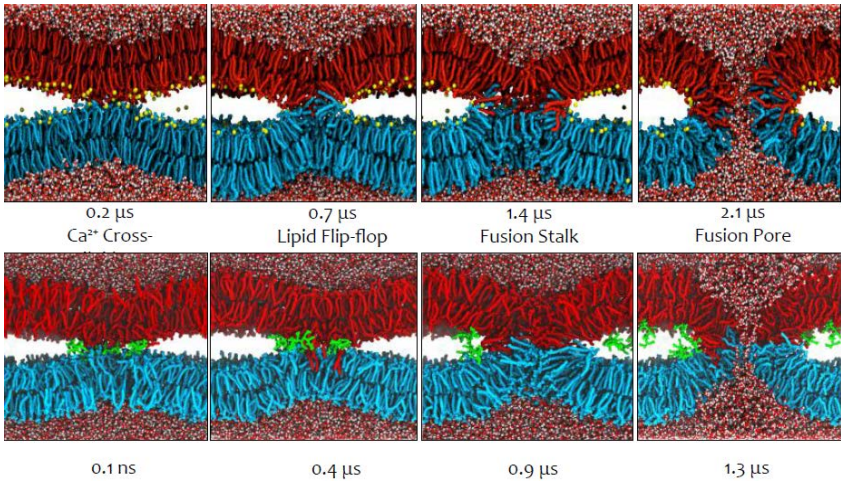


Figure 1.4: Fully atomistic molecular simulations demonstrating that calcium ions and nona-arginines induce membrane fusion by the same mechanism involving lipid cross-linking and flip-flop, followed by creation of a fusion stalk and pore.

phosphatidylethanolamine-rich vesicles, which until then had only been suggested indirectly based on experiments and coarse-grained simulations. Moreover, in the same study Jungwirth's group with collaborators found that from the mechanistic point of view the passive membrane penetration of arginine-rich peptides is analogous to calcium-induced membrane fusion. A hypothesis has been formulated that passive cell entrance of arginine-rich peptides may proceed via a hitherto unexplored mechanism involving induction of membrane budding and multilamellarity, followed by a fusion process analogous to calcium-induced membrane fusion. In general, it has been suggested that membrane properties such as curvature, lipid composition, and presence of domain boundaries may control fusion in the presence of elevated concentrations calcium ions or cationic arginine-rich peptides. Additionally membrane domains and their boundaries with strong curvature have been shown to be important sites for fusion.

Ad (ii), structure, dynamics, and reactivity of solvated electrons: Jungwirth's group has been a leader in the quest of establishing the molecular structure, dynamics, and reactivity of solvated electrons. With their characteristic blue color originating from dissolving sodium in liquid ammonia, which were first observed already in the 19th century, solvated electrons remain to be enigmatic species. This is despite their considerable practical importance, e.g., as the key agents in the industrially relevant Birch reduction process for hydrogenation of aromatic hydrocarbons. Solvated electrons are also invoked in specific mechanisms of radiative damage of DNA, which is being exploited, e.g., in radiative cancer therapy.
Recent computational work from Jungwirth's lab, based primarily on state-of-the-art ab initio molecular dynamics simulations, led to a firm establishment of structural and energetic properties of a single electron in water. Moreover, their laboratory experiments following the explosive and non-explosive regimes of the reaction of alkali metals in water allowed for establishing a Coulomb explosion mechanism as the key behind the explosive behavior, as well as to observe the transient electrons solvated in water by a naked eye. Having this vigorous reaction under control has opened a new way of massive doping of electrons to water and thus reaching hitherto unexplored high concentration regimes where dielectrons are formed and water may even exhibit metallic behavior. Combined with the liquid microjet

photoelectron spectroscopy co-developed with researchers at the BESSY II synchrotron in Berlin has opened a qualitatively new way to explore and characterize these fascinating systems. Currently, both experimental and computational tools are being developed and sharpened on systems, where high concentrations of solvated electrons can be reached without the danger of explosion, i.e., for alkali metal-liquid ammonia mixtures.

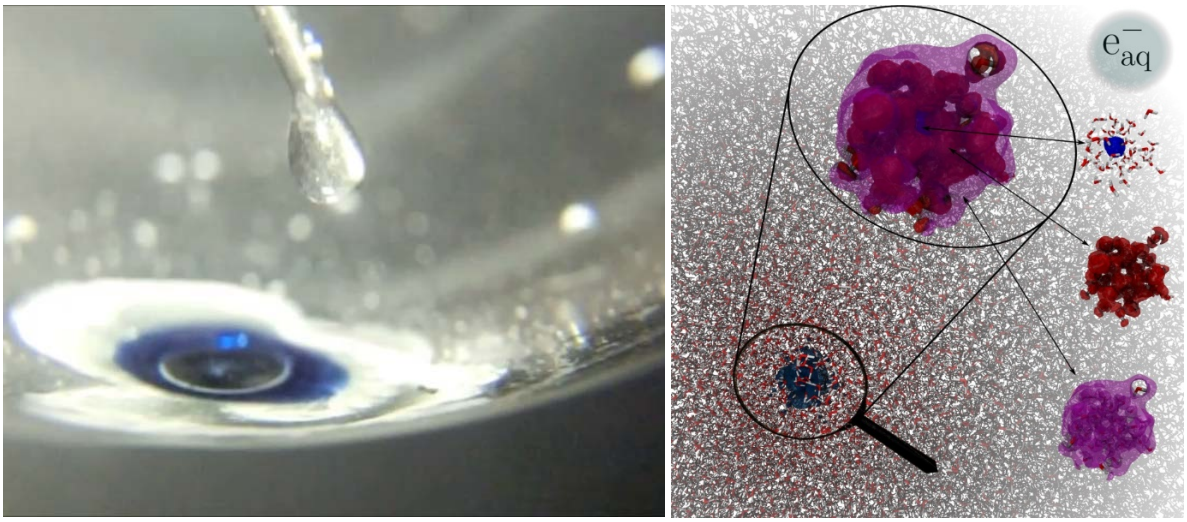


Figure 1.5: Experimental and computational investigation of hydrated electrons.

Ad (iii), d**evelopment of force fields for molecular simulations with implicit inclusion of electronic polarization:** Jungwirth's group has been strongly involved in developing accurate interaction potentials (force fields) for the description of interactions of ions in biologically relevant aqueous environments. Charged peptides and ions, such as calcium o rmagnesium, play a key role in many physiological processes. Understanding at the molecular level these processes requires elucidating how, e.g., calcium binds to specific protein sites, how it triggers conformational changes, and what is the mechanism of its unbinding. Molecular dynamics simulations have proven to be a powerful method to complement experimental tools, as they allow to interpret experimental data by giving access to molecular details of the investigated processes. However, standard non-polarizable force fields typically employed in simulations suffer from strong overbinding artifacts especially for divalent ions interacting with biomolecules, including proteins, phospholipids, and nucleic acids. This artifact dramatically affects the behavior of virtually all simulated ion-containing biological systems, and thus need to be fixed in order to avoid the uncomfortably common Garbage-In-Garbage-Out (GIGO) scenario. The above overbinding artefact originates primarily from the lack of electronic polarization in commonly used non-polarizable force fields, which would further screen the interaction between two charged moieties. It can be shown that electronic polarizability can be rigorously included in a mean-field way into non-polarizable force fields by simply scaling the ionic charges by the inverse of the square root of the high-frequency dielectric constant of the system (i.e., by ~0.75, for water and similar systems). Following an initial suggestion in the literature, Jungwirth's group has further developed and extensively tested this mean-field approach, denoted as the Electronic Continuum Correction (ECC). It has been demonstrated that this approach allows to properly describe ion pairing and ion binding in biological systems at no extra computational cost.

Since the introduction of the ECC framework, Jungwirth's group has applied and tested it systematically, starting from simple salt solutions made of monoatomic monovalent and divalent ions (*e.g.* LiCl and $CaCl_2$). Next, investigations were extended the studies to more complex molecular ions (*e.g.* acetate and sulfate) and charged moieties of biomolecules such as lipids and proteins. Simulation results were favorably compared to experimental data –

neutron scattering for salt solutions, or NMR and electrophoresis for biomolecules – and to advanced *ab initio* simulations. It has been have shown that the use of ECC qualitatively improves the ion binding properties in all the studied systems, which opens way for realistic simulations of complex ion-containing biological systems, such as those involving calcium, proteins/peptides, and lipid membranes in signalling pathways.

Selected key publications (Jungwirth group): (J1) Mason *et al.*: Quantifying the Strength of a Salt Bridge by Neutron Scattering and Molecular Dynamics. *J. Phys. Chem. Lett.* **2015**, *10*, 3254-3259. (J2) Buttersack *et al.*.: Valence and Core-Level X-ray Photoelectron Spectroscopy of a Liquid Ammonia Microjet. *J. Am. Chem. Soc.* **2019**, *141*, 1838-1841. (J3) Kirby *et al.*: Charge Scaling Manifesto: A Way of Reconciling the Inherently Macroscopic and Microscopic Natures of Molecular Simulations. *J. Phys. Chem. Lett*. **2019**, *10*, 7531-7536. (J4) Allolio *et al.*: Arginine-rich cell-penetrating peptides induce membrane multilamellarity and subsequently enter via formation of a fusion pore. *Proc. Nat. Acad. Sci.* **2018**, *115* 11923-11928. (J5) Vazdar *et al.*: Arginine "Magic": Guanidinium Like-Charge Ion Pairing from Aqueous Salts to Cell Penetrating Peptides. *Acc. Chem. Res.* 2018, 51, 1455-1464. (J6) Duboue-Dijon *et al.*: Hydration and Ion Pairing in Aqueous Mg^{2+} and Zn^{2+} Solutions: Force-Field Description Aided by Neutron Scattering Experiments and Ab Initio Molecular Dynamics Simulations. *J. Phys. Chem. B* **2018**, *122*, 3296. (J7) Martinek *et al.*: Calcium Ions in Aqueous Solutions: Accurate Force Field Description Aided by Ab Initio Molecular Dynamics and Neutron Scattering. *J. Chem. Phys.* **2018**, *148*, 222813. (J8) Tesei *et al.* Self-Association of a Highly Charged Arginine-Rich Cell-Penetrating Peptide. *Proc. Nat. Acad. Sci.* **2017**, *114*, 11428-11433. (J9) Timr *et al.*.: Membrane Binding of Recoverin: From Mechanistic Understanding to Biological Functionality. *ACS Central Science* **2017**, *3*, 868-874. (J10) Palivec *et al*.: Computational and Structural Evidence for Neurotransmittermediated Modulation of the Oligomeric States of Human Insulin in Storage Granules. *J. Biol. Chem.* **2017**, *292*, 8342-8355. (J11) Bilkova *et al.*: Calcium Directly Regulates Phosphatidylinositol 45-Bisphosphate Headgroup Conformation and Recognition. *J. Am. Chem. Soc.* **2017**, *139*, 4019-4024. (J12) Okur *et al*.: Beyond the Hofmeister Series: Ion Specific Effects on Proteins and Their Biological Functions. *J. Phys. Chem. B* **2017**, *121*, 1997-2014. (J13) Heyda *et al*.: Guanidinium can both Cause and Prevent the Hydrophobic Collapse of Biomacromolecules. *J. Am. Chem. Soc.* **2017**, *139*, 863-870. (J14) Mason *et al*.: A Non-Exploding Alkali Metal Drop on Water: From Blue Solvated Electrons to Bursting Molten Hydroxide. *Angew. Chem.* **2016**, *55*, 13019-13022. (J15) Melcr *et al*.: Transmembrane Potential Modeling: Comparison between Methods of Constant Electric Field and Ion Imbalance. *J. Chem. Theor. Comput.* **2016**, *12*, 2418-2425. (J16) Allolio *et al*.: Guanidinium Pairing Facilitates Membrane Translocation. *J. Phys. Chem. B* **2016**, *120*, 143-153. (J17) Pluhařová *et al.*: Modeling Photoionization of Aqueous DNA and its Components. *Acc. Chem. Res*. **2015**, *48*, 1209-1217. (J18) Kohagen *et al*.: Exploring Ion-Ion Interactions in Aqueous Solutions by a Combination of Molecular Dynamics and Neutron Scattering. *J Phys. Chem. Lett.* **2015**, *6*, 1563-1567. (J19) Mason *et al.*: Coulomb Explosion during the Early Stages of the Reaction of Alkali Metals with Water. *Nature Chem*. **2015**, *7*, 250-254. 262. (J20) Kulig *et al.*: Experimental Determination and Computational Interpretation of Biophysical Properties of Lipid Bilayers enriched by Cholesteryl Hemisuccinate. *Biochim. Biophys. Acta Biomembranes* **2015**, *1848*, 422-432. (J21) Pluhařová *et al.*: Oxidation Half-Reaction of Aqueous Nucleosides and Nucleotides via Photoelectron Spectroscopy Augmented by Ab Initio Calculations. *J. Am. Chem. Soc.* **2015**, *137*, 201-209.

The Rulíšek's group research in the 2015-2019 period revolved around four main topics: (i) theoretical (bio)inorganic chemistry; (ii) molecular properties of endohedral fullerenes (EMFs); (iii) computational electrochemistry; (iv) physico-chemical principles of protein structure ("ab initio protein folding).

Ad (i), theoretical (bio)inorganic chemistry: In 2015, in a joint study with Prof. Ulf Ryde, we accomplished our work on multi-copper oxidases. [R1] The complete reaction cycle has been

conceived and most of its parts confirmed experimentally. We have also continued our work on the challenging non-heme diiron metalloenzymes, mostly □^{9}-desaturase. In collaboration with the experimental group of Prof. Roithová, the magnitude of aurophilic interactions was determined by concerted experimental and computational efforts. [R2] For almost three decades, attractive metallophilic (aurophilic, argentophilic, cuprophilic, …) interactions were shown to play an important role in arrangement and stabilization of oligonuclear metal ion complexes. In our study, we reported a combined experimental and theoretical assessment of aurophilic interactions in closed-shell gold(I) dimers. The experimental binding energies were obtained for charged [(LH)AuCl]$^{+}$…[(L')AuCl] dimers (L is either a phosphine or a *N*-heterocyclic carbene ligand) in the gas phase. These energies served for benchmarking of correlated quantum chemical calculations (CCSD(T)-calibrated SCS-MP2/CBS method) that were then applied to neutral [(L)AuCl]…[(L')AuCl] dimers. The overall attractive interactions between monomeric units are in the order of 100–165 kJ mol^{-1} in the charged dimers and of 70–105 kJ mol^{-1} in the corresponding neutral dimers. In the neutral dimers, pure aurophilic interactions account for 25-30 kJ mol^{-1}, the dipole-dipole interactions for 30–45 kJ mol^{-1}, and the L…L' "inter-ligand" dispersion interactions for 5-25 kJ.mol^{-1}. Energy of the aurophilic interactions is thus comparable or even larger than that of strong hydrogen bonds. In a study carried out in parallel, some of these complexes (1,2,4-Triazole-based *N*-heterocyclic carbene complexes of gold (I)) were tested for biological activity [R3] since there has been recently some interest in employing gold in various therapies (cancer, rheumatic arthritis, …). Last but not least we have discovered unprecedented Au(I)…$^{+}$H-N weak interactions, both experimentally and theoretically. [R4]

Ad (ii), molecular properties of endohedral fullerenes (EMFs): We studied rectification properties of EMFs for molecular electronics and exotic chemical bonds in endohedral fullerenes. We have shown in pilot study that endohedral fullerenes with a dipolar molecule enclosed, MX@C_{70} behave as electric field-driven single-molecule switching diodes and memristors. [R5] We have also computationally characterized the first thorium fullerene, Th@C_{84}. [R6] Shortly after our study, a number of new Th EMFs have been synthesized and characterized experimentally. In 2015, we have predicted a new kind of *unwilling* U–U bonding in U_2@C_{80} and analogous systems. Our prediction was confirmed in 2018, when U_2@C_{80} with single U–U bond was characterized experimentally. Fullerene cages can also host molecular complexes. A system with HF•H_2O enclosed in C_{70} has been reported in 2017. The reported rather short FH···OH_2 contact led us to study the chemical bonding between enclosed neutral molecules and fullerenes. We have shown that the short F···H contact in HF•H_2O@C_{70} is not a mere compression of molecules inside the cage as anticipated in the experimental study. We described a new type *charge-shifting* bonding in which LP-π bonding between the electron lone-pairs of the enclosed molecules and the fullerene cage (caused by the curvature of the π system of the cage) is utilized. [R7] In a follow up study, we calculated F_2 in C_{60}, which turned out to be F_2^-@C_{60}^+, the first system, where enclosed moiety is negative and fullerene cage positive. Notably, the interactions between enclosed F_2^- and cage were found to be of purely electrostatic nature making this molecule the first *single-molecule crystal* ever. A characteristic of F_2^-@C_{60}^+ and analogous systems is that they can accommodate electrons, which affects the bond length of the enclosed X_2, and ultimately may turn the enclosed cluster into two X^- anions.[R8] This might be a route towards a new class of molecular transistors.

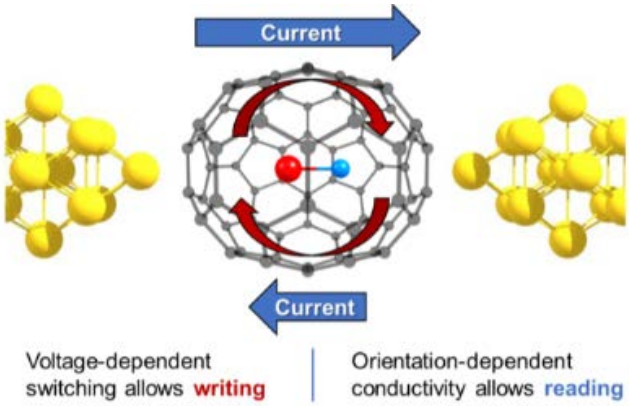


Figure 1.6: In MX@C_{70} memristor, high voltage turns around dipolar MX molecule (encodes information), which changes conductivity of the system (enables reading of information).

Ad (iii), computational electrochemistry: In a collaboration with the group of Dr. Martin Srnec (Institute of Physical Chemistry, CAS; until 2019 also part-time in the Rulíšek group) we devised an elegant protocol denoted variable temperature H-atom addition/abstraction (VTHAA) method [R9]. It has been shown that the VTHAA method (protocol) yield accurate reduction potentials of the highly charged species by calculating the data for their less charged cognates. The protocol has been also successfully applied to a series of 47 electrochemically characterized [Fe(X)$_n$(O)] complexes which were considered by many as one of the most challenging systems for the computations of redox potentials [R10]. This all led to a joint study (with Dr. Srnec) that has highlighted the role of asynchronicity in the proton and electron transfer in hydrogen-atom transfer (HAT) reactions.[R11] It has been shown that the newly defined asynchronicity factor is intimately related with the reorganization energy in the Marcus equation of electron transfer and thus we provided explanation of the computed and experimentally observed phenomenon: “asynchronous HAT reactions are faster that their synchronous counterparts” (c.f. Figure 1.7). This concept has gained considerable attention in the chemical community and was soon afterwards proved experimentally (Prof. John Anderson, Univ. Chicago).

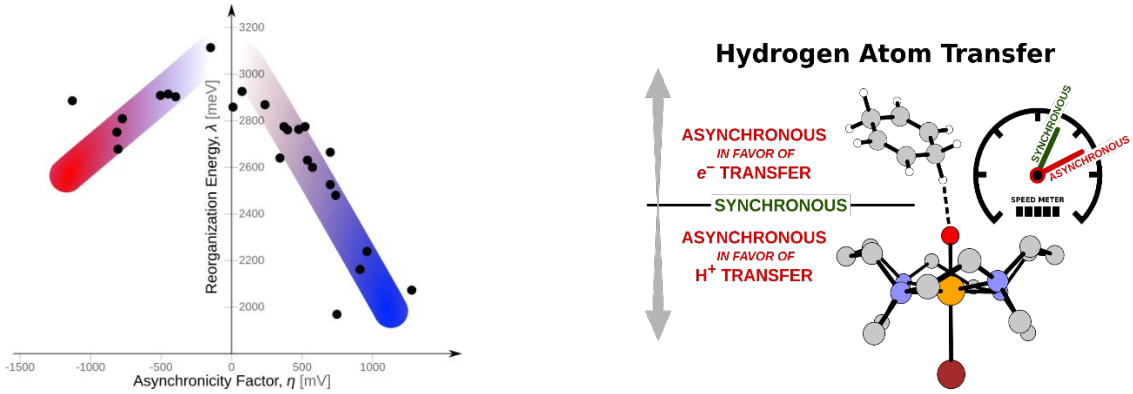


Figure 1.7: Correlation between the asynchronicity factor and reorganization energy (left). Higher asynchronicity in the proton and electron transfer implies faster HAT reaction for the same overall thermodynamics (right).

Ad (iv), physico-chemical principles of protein structure (“ab initio protein folding): The aim of the project was the detailed and exhaustive mapping of the complex conformational space of short peptides and investigation of the relation between the variability of this space

and protein folding. This involved the development and calibration of an efficient and robust protocol ensuring that all relevant local minima on the free energy surface of medium-sized molecules (100-200 atoms) were identified. [R12] The protocol was based on accurate, yet efficient quantum chemical methods and an advanced solvation method, such as COSMO-RS. [R13] The conformational space of the tripeptides composed of 20 naturally occurring amino acids (8000 systems) was computed, catalogued, and analysed. We have shown that there is a correlation between secondary structures preferentially adopted by a particular tripeptide, measured by its occurrence/frequency in the protein three-dimensional structures (taken from the Protein Data Bank) and its conformational preference for extended vs. helical conformation predicted by quantum chemical calculations. [R14]. For a particular protein - WW domain – we have shown that there is also a correlation between conservation of certain fragments (by evolution) and their intramolecular interaction energies. [R15] At the same time, we showed that parts of the protein protein chain that are close to their (free) energy minima (i.e. *are unstrained*), might have been used by evolution as initiators of the folding process. Finally, taking a full advantage of a large and complete set of (presumably accurate) "ab initio" data we will compare them with the existing folds in proteins which may ultimately answer the fundamental question: ”How much of the protein structure is encoded in its building blocks?"

Selected key publications (Rulíšek group): (R1) Li *et al.*: Catalytic Cycle of Multicopper Oxidases Studied by Combined Quantum- and Molecular-Mechanical Free-Energy Perturbation Methods. *J. Phys. Chem. B* **2015**, *119*, 8268–8284. (R2) Andris *et al.:* Aurophilic Interactions in [(L)AuCl]…[(L')AuCl] Dimers: Calibration by Experiment and Theory. *J. Am. Chem. Soc.* **2018**, *140,* 2316-2325; (R3) Turek *et al.*: 1,2,4-Triazole-based *N*-heterocyclic carbene complexes of gold (I): synthesis, characterization and biological activity. *Appl. Organometal. Chem.* **2016**, *30*, 318-322; (R4) Straka *et al.*: Spectroscopic and Computational Evidence of Intramolecular Au···H^+–N Hydrogen Bonding. *Angew. Chem. Int. Ed.* **2019**, *58*, 2011-2016; (R5) Jaroš *et al.*: Fullerene-Based Switching Molecular Diodes Controlled by Oriented External Electric Fields. *J. Am. Chem. Soc.* **2019**, *141* (50), 19644–19654; (R6) Kaminský *et al.*: Properties of the Only Thorium Fullerene, Th@C_{84}, Uncovered. *J. Phys. Chem. A* **2017**, *121* (16), 3128–3135.; (R7) Jaroš *et al.*: How Does a Container Affect Acidity of Its Content: Charge-Depletion Bonding Inside Fullerenes. *Chem. – Eur. J.* **2018**, *24* (17), 4245–4249.; (R8) Foroutan-Nejad *et al.*: Buckyball Difluoride F_2^-@C_{60}^+—A Single-Molecule Crystal. *Angew. Chem. Int. Ed.* **2018**, *57* (42), 13931–13934; (R9) Bím *et al.*: Accurate Prediction of One-Electron Reduction Potentials in Aqueous Solution by Variable-Temperature H-Atom Addition/Abstraction Methodology. *J. Phys. Chem. Lett.* **2016**, *7*, 7-13; (R10) Bím *et al.*: Computational Electrochemistry as a Reliable Probe of Experimentally Elusive Mononuclear Nonheme Iron Species. *J. Phys. Chem. C* **2018**, *122*, 10773-10782; (R11) Bím *et al.*: Beyond the Classsical Thermodynamic Contributions to Hydrogen Atom Abstraction Reactivity. *Proc. Natl. Acad. Sci.* **2018**, *115*, E10287-E10294; (R12) Gutten *et al.*: Macrocycle Conformational Sampling by DFT-D3/COSMO-RS Methodology. *J. Chem. Inf. Model.* **2018**, *58*, 48-60; (R13) Řezáč *et al.*: Toward Accurate Conformational Energies of Smaller Peptides and Medium-Sized Macrocycles: MPCONF196 Benchmark Energy Data Set. *J. Chem. Theor. Comput.* **2018**, *14*, 1254–1266; (R14) Culka *et al.*: Toward *Ab Initio* Protein Folding: Inherent Secondary Structure Propensity of Short Peptides from the Bioinformatics and Quantum-Chemical Perspective. *J. Phys. Chem. B* **2019**, *123*, 1215–1227; (R15) Culka *et al.*: Factors stabilizing β-sheets in protein structures from a quantum-chemical perspective. *J. Phys. Chem. B* **2019**, *123*, 6453-6461.

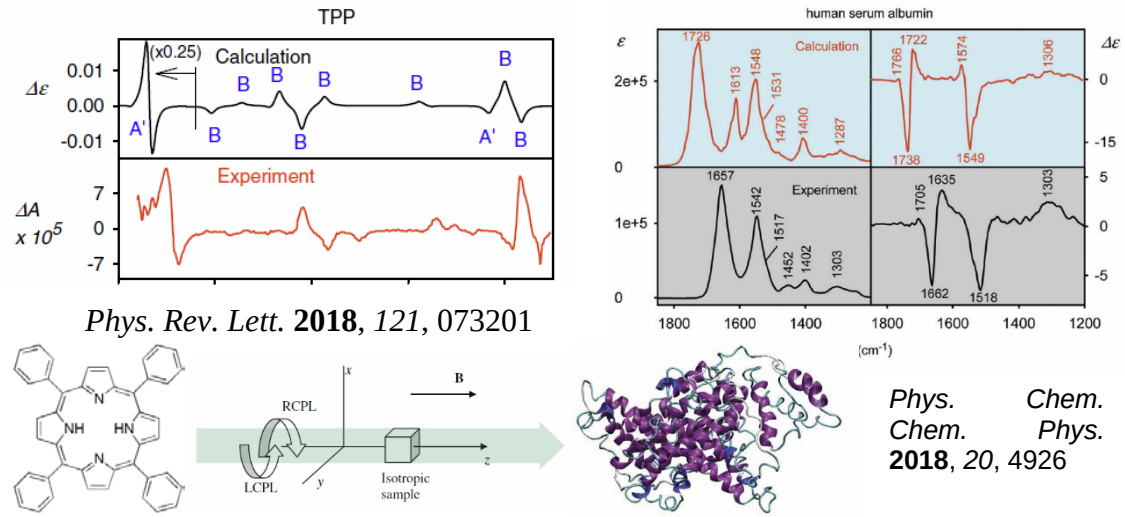
The Lazar group has been focusing on technical improvements to the technique of two-photon polarization microscopy (2PPM) previously developed by the laboratory, and on developing new applications of this technique. We applied the technique to elucidation of the nature of interactions between G-proteins and G-protein coupled receptors in the resting state, [L1] as well as to interactions between potassium transporters and other proteins. [L2] We have put

considerable effort into developing the ability to use 2PPM for insights into structure of membrane proteins in living cells. Towards this goal we verified the ability of the technique to determine molecular orientations with respect to lipid membranes using a simple synthetic model system. [L3] In following work, we developed a cellular system allowing making such determinations in proteins, and we demonstrated its usability. A major effort was invested in determining the directionality of optical properties of fluorescent proteins. The results of this work are expected to find a wide range of uses by a large number of researchers. The uses will include interpretations of fluorescence energy transfer measurements, of polarization microscopy observations, but also of single molecule observations. The team has demonstrated the ability of 2PPM to observe effects of molecular orientations in probes expressed in living cells from endogenous promoters, that is, expressed at the low concentrations of endogenously expressed proteins of interest (work in progress). In order to allow rapid development of genetically encoded probes, the group has re-engineered the green fluorescent protein in a fashion that should allow predictable exhaustive sampling of orientations at which the fluorescent protein is inserted into proteins of interest (work in progress). The work of the team is highly multidisciplinary, novel, and requires overcoming numerous challenges.

Selected key publications (Lazar group): (L1) Bondar et al.: The G protein Gi1 exhibits basal coupling but not preassembly with G protein-coupled receptors. *J Biol Chem* **2017,** *292*, 9690-9698. (L2) Smidova *et al.*: The activity of Saccharomyces cerevisiae Na(+), K(+)/H(+) antiporter Nha1 is negatively regulated by 14-3-3 protein binding at serine 481. *Biochim Biophys Acta Mol Cell Res* **2019,** *1866*, 118534. (L3) Timr *et al.*: Nonlinear Optical Properties of Fluorescent Dyes Allow for Accurate Determination of Their Molecular Orientations in Phospholipid Membranes. *J Phys Chem B* **2015,** *119* (30), 9706-16.

Research activity and characterisation of the main scientific results

The Bouř group of **Biomolecular Spectroscopy** continued to develop theoretical and experimental tools for biomolecular spectroscopy, in particular vibrational optical activity. A way was found to efficiently calculate magnetic circular dichroism and the technique was applied to a range of systems including fullerenes and aromatic hydrocarbons. Similarly, in the collaboration with the University of Illinois at Chicago, Prof. T. A. Keiderling, common density functional theory was used to interpret vibrational circular dichroism (1). New physical phenomena were discovered, related to resonance Raman optical activity of gasses in the magnetic field (2).
A very useful application appeared for the Raman optical activity instrument, capable of detection weal lanthanide circularly polarized luminescence. Lanthanide probes were shown to sensitively react on the environment and detect biomolecules, such as sugars, peptide, and nucleic acids (3). In collaboration with the University of Edmonton, Prof. Y. Xu, we for the first time could explain and predict induced Raman chirality in organic solvents, potentially useful for analysis and imaging (4). The group has a particularly fruitful collaboration with Palacký University Olomouc, Dr. J. Kapitán, a recognized expert in chiral Raman spectroscopy. New computer programs were developed for understanding vibrational properties of biopolymers (5). Other activities involve collaborations with Osaka University (Prof. S. Yamamoto, Raman spectroscopy), Tokushima Bunri University (Prof. Y. Tanaka, NMR of nucleic acids), organic synthesis, and research into molecular basis of protein misfolding diseases.



Phys. Rev. Lett. **2018**, *121*, 073201

Phys. Chem. Chem. Phys. **2018**, *20*, 4926

Research in Biomolecular Spectroscopy: Developed theories made it possible to predict molecular vibrational circular dichroism (left) and vibrational optical activity spectra of giant proteins molecules (right).

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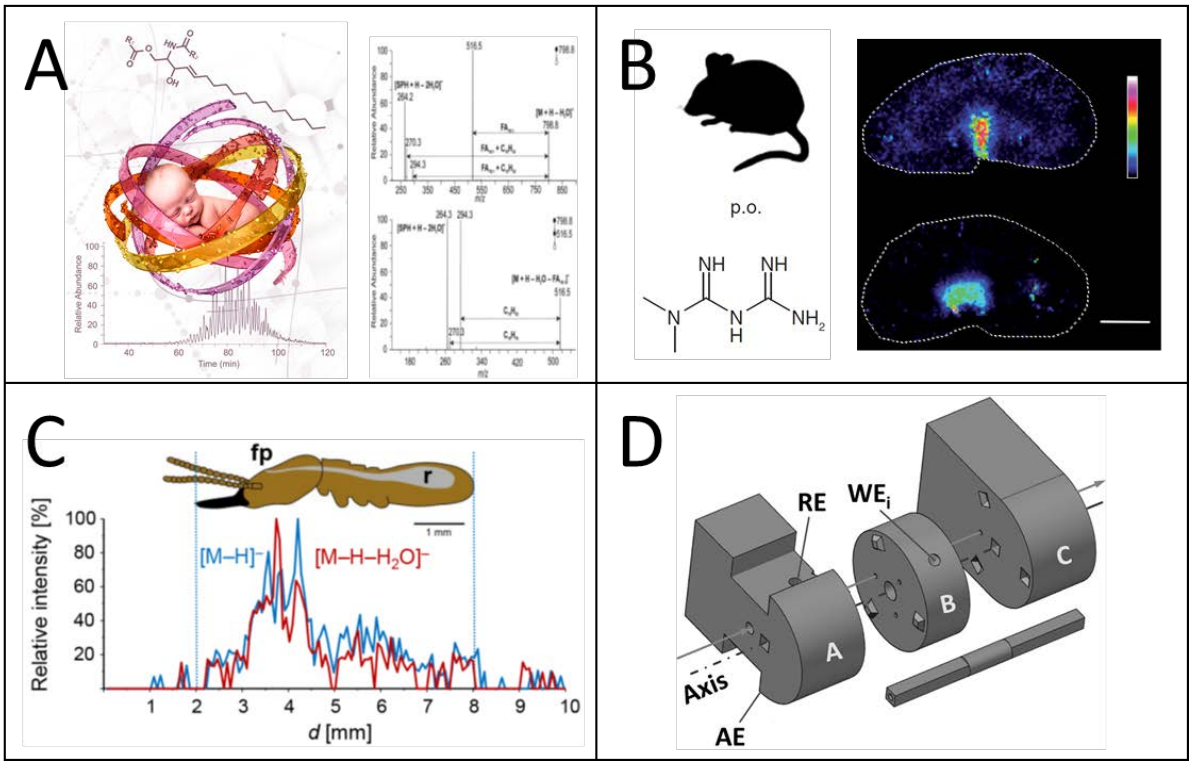
A complete list of publications can be found in ASEP.

The Cvačka group of **Mass Spectrometry** continued to work in several directions including structural analysis and spatial distribution of lipids in biological samples and tissues, development of new ion sources for gas-phase ionizations, or electrochemical generation of metal cations for adduct formation in electrospray ionization. Numerous collaborations with other researchers within and outside the IOCB resulted in many papers where mass spectrometry contributed to important findings.

For a long time, the group has been striving to disclose the lipid composition of vernix caseosa with the aim to use its synthetic substitutes in medicine. Vernix caseosa is a biofilm formed on the skin of a human foetus during the last trimester of pregnancy and it has interesting antimicrobial and wound healing properties. Systematic studies including in-depth chromatographic fractionation and mass spectrometry of vernix caseosa made it possible to discover and/or characterize three lipid classes including diesters of 1,2-diols (1), nonhydroxylated 1-O-acyleceramides (2), and cholesteryl esters of omega-(O-acyl)-hydroxy fatty acids (3). A mass-spectrometry based method for localization of double bonds was developed and applied to triacylglycerols (4). A new protocol for MALDI mass spectrometry imaging (MALDI-MSI) of lipids in the brains of mouse models of neurodegeneration was developed. Using 1,5-diaminonaphthalene matrix and APP/PS1 mouse model at an age of 6 months, a colocalization of amyloid β plaques with different phospholipids, sphingolipids, and lysophospholipids was found (5). MALDI-MSI was also used to monitor the pharmacokinetics of metformin in mice and this approach was compared to traditional approaches based on LC/MS (6).

Gas-phase ionizations are important in mass spectrometry of many organic compounds including lipids and various natural products. The group investigated the applicability of atmospheric-pressure photoionization (APPI) for the detection of such compounds directly from sample surfaces (ambient mass spectrometry). New ion sources have been developed to desorb the analytes from flat, nearly-flat, or uneven surfaces. They were used for detecting analytes on TLC plates (7), studying the spatial distribution of defense compounds on insects (8), and for detecting analytes from various small 3D objects including food and pharmaceutical samples (9). The research was done in close collaboration with the IOCB Development Workshops and yielded unique technical solutions including a computer-controlled movement of the sample or laser trigonometry for recording the sample morphology. Besides ambient applications, the group has been interested in the applicability of atmospheric pressure chemical ionization (APCI) and photoionization in LC/MS at very low flow rates. This research is motivated by the fact that there is no commercial ion source capable of ionizing liquid samples at microliter-per-minute (or lower) flow rates using APCI or APPI. Such ion sources would make it possible to couple these ionization techniques to micro-and nano-HPLC with all the benefits including higher sensitivity and low sample consumption (similar to nanoelectrospray, a miniaturized version of electrospray used in proteomics). Two different ion sources have been developed in the group: (i) an ion source based on heated glass microchip provided by Finnish collaborators (10), and (ii) an ion source with a tubular nebulizer, which can be easily assembled from common chromatographic fittings (11).

The most frequently used ion sources for LC/MS are based on electrospray ionization, which generates protonated or deprotonated molecules as the main ions. Such ionization is widely applicable; however, certain analytes are difficult to ionize and metal adduct formation can make their detection highly sensitive. Moreover, metal adducts fragment in a different way than protonated molecules and can thus generate unique structural information. Therefore, an electrochemical device with three different solid electrodes (Ag, Au, and Cu) was developed and hyphenated with electrospray mass spectrometry (12). The device was patented (13).



Research in Mass Spectrometry: A, Cover page of *J. Lip. Res*. highlighting the discovery and characterization of nonhydroxylated 1-O-acyleceramides in vernix caseosa and mass spectra of these lipids (2); **B**, MALDI imaging of metformin in mouse kidney (6); **C**, ion traces of (*E*)-1-nitropentadec-1-ene recorded along the median line of *P. simplex* termite soldier (8); **D**, Scheme of the electrochemical device containing three working electrodes, an auxiliary electrode and a reference electrode (12, 13).

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A complete list of publications can be found in ASEP.

The Kašička group of **Electromigration Methods** has focused the research activities on three areas, instrumentation, methodology, and applications of high-performance capillary electromigration (CE) methods.

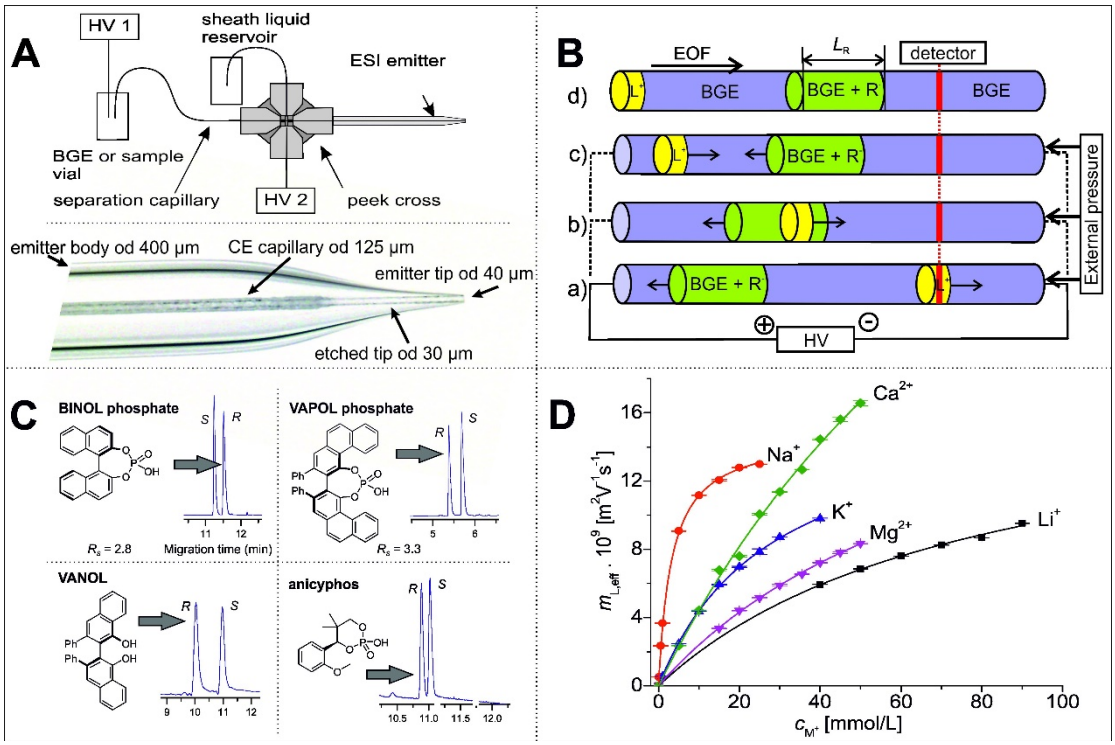
Two new types of interfaces for the on-line coupling of home-made CE devices with electrospray ionization mass spectrometry (ESI/MS) detection have been developed: sheathless porous tip and nano-sheath liquid flow CE-ESI/MS interfaces (1). Both interfaces were successfully applied for the investigation of molecular interactions, particularly for the determination of stability constants of potassium complexes with crown ethers by affinity CE.

The group also designed and constructed a new type of fully automated two-dimensional (2D) capillary electrophoretic (2D-CE) analyser with a multidimensional detection system. It consists of two in-series connected fused silica capillaries via a cross-shaped interface, which enables independent filling of the two capillaries by different separation media. The multidimensional detection system consists of a universal contactless conductivity detector, UV-vis absorption detector or fluorescence detector. Two-dimensional separation system is based on the combination of orthogonal separation principles, such as isotachophoresis, isoelectric focusing or micellar electrokinetic chromatography in the first dimension and zone electrophoresis in a free solution or sieving media or affinity electrophoresis in the second dimension. The functionality of the device was verified but its practical application for analysis of complex samples has yet to be implemented.

In the areas of methodology and applications, several CE methods, particularly capillary zone electrophoresis (CZE), affinity capillary electrophoresis (ACE), capillary isotachophoresis (CITP), micellar electrokinetic chromatography (MEKC) and open-tubular capillary electrochromatography (OT-CEC) were developed and applied for analysis and physicochemical characterization of (bio)molecules.

CZE in a series of aqueous background electrolytes in a wide pH range (2.0-12.0) has been applied for determination of acid-base and electromigration properties of 5-azacytosine derivatives, potential new antiviral and anticancer drugs (2) and the estimation of acidity constants and actual ionic mobilities of insect antimicrobial peptides, potential new antibiotics (3). Newly developed pressure-assisted CZE method enabled an accelerated determination of acidity constants of environmentally important compounds, triazole fungicides (4).

The combination of CITP and CZE allowed the determination of effective charge of strongly basic polycationic antimicrobial peptides (5). Newly developed methods, partial filling ACE (PF-ACE) and pressure-assisted PF-ACE were employed for investigation of enantioselective interactions of helquats, a new class of functional organic molecules, with chiral acidic aromatic analytes (drugs and catalysts) (6) and for the quantitative evaluation of the strength of the non-covalent binding of human insulin with biologically relevant ligands, dopamine, serotonin, arginine, and phenol (7). Classical mobility shift ACE with cyclodextrin-based chiral selectors has been employed for i) separation of enantiomers of diquats, a new type of functional organic molecules (8), ii) separation of a new type of stereoisomers, rotamers of 5-nitrosopyrimidines (9), iii) chiral analysis of polypyridyl heavy metal complexes (10), and iv) study of non-covalent interactions of cyclic peptides with univalent and divalent metal cations (11). In addition, the cocktail effect of tebuconazole, resveratrol, and copper on essential enzymes was studied (12).



Electromigration Methods achievements: A, Interface for CE-ESI-MS and detail of the nanospray emitter (1); **B**, Experimental setup of partial filling affinity CE for determination of binding constant of biomolecular complexes (7); **C**, chiral separations with diquat chiral selector (8); **D**, Binding constant determination of cyclic peptide complexes with selected cations (11).

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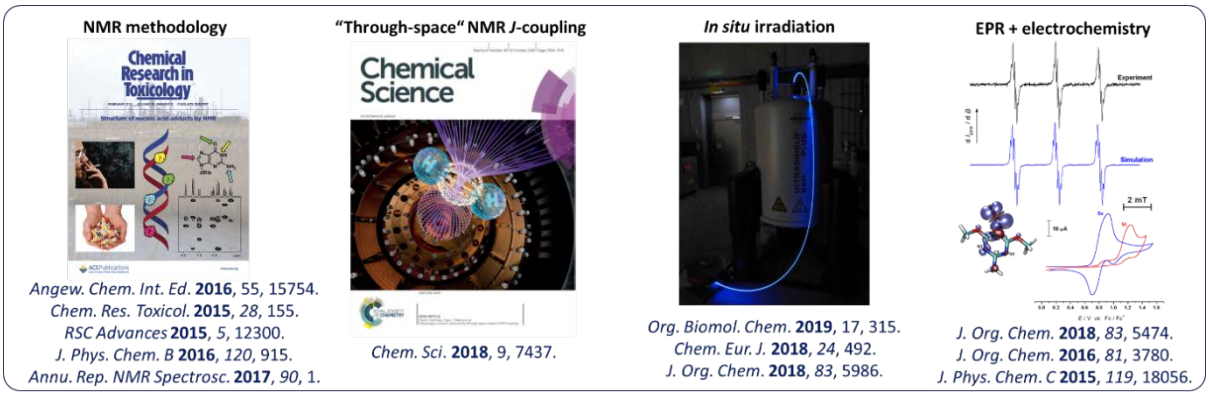
A complete list of publications can be found in ASEP.

The Šaman group of **NMR Spectroscopy** deals with many aspects of experimental NMR/EPR spectroscopy of solutions and solids, molecular modelling, and theoretical calculations of spectroscopic parameters. We have applied both experimental and theoretical methods in many studies of the structure and properties of biologically active compounds (e.g. modified components of nucleic acids), of intra- and inter-molecular interactions (particularly hydrogen bonding), and of reaction mechanisms.

The NMR group develop and investigate new spectroscopic methods. For example, they have discovered that, in contradiction to many textbooks, 'through-space' J-couplings between hydrogen atoms not connected by covalent bonds can be detected and used for structure determination. The observation of the 'through-space' J-coupling between hydrogen atoms called for a new interpretation of the chemical bonding phenomenon. The paper was highlighted by the front cover in Chemical Science (1). We also implemented a methodology for *in situ* irradiation during NMR experiments, which enables NMR investigation of photochemical processes (2).

With the help of EPR spectroscopy, the group investigate organic radicals and transitional metal complexes. The EPR was successfully applied in material science (e.g. studies on nanodiamods and the triazine-based graphitic carbon nitrides) as well as in studies of reaction (redox) mechanism using EPR photochemistry and a combination of EPR spectroscopy with electrochemistry. The latter involved studies on natural products like flavins and flavonoids as well as newly synthesized nitroso compounds, extended pyridinium derivatives and diamino-naphtoquinone derivative, which is considered to be a promising candidate for singlet-fission process in solar cells (3).

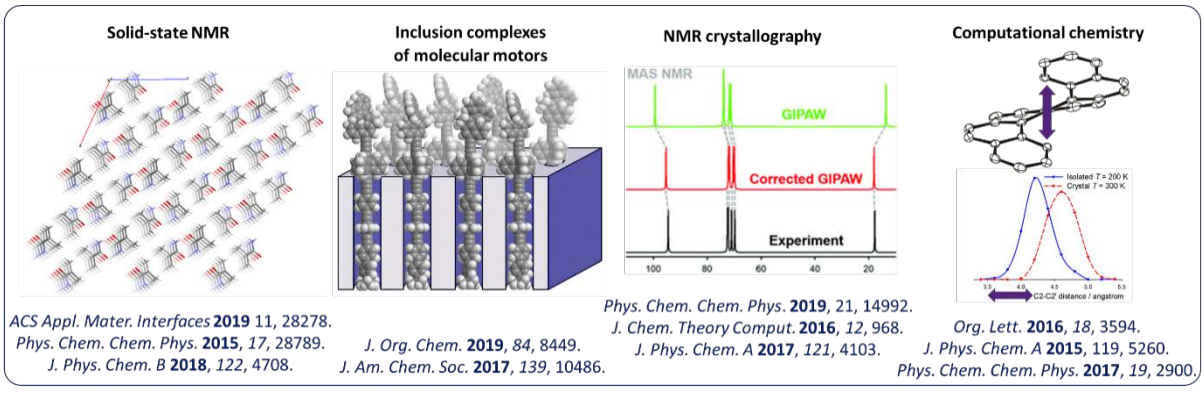


Research in NMR Spectroscopy: Methodology development

One of the spectrometers is dedicated to measurements of solid-state NMR (SS-NMR) experiments. This spectrometer is equipped with a unique ultra-fast magic-angle spinning probe (up to 80 kHz spinning speed, rotor outer diameter of 1 mm) allowing high-resolution ^{1}H NMR experiments and proton-detected SS-NMR experiments. Using this equipment, the NMR Spectroscopy Group investigated, for example, polymorphic transformation of drugs induced by glycopolymeric vesicles designed for anticancer therapy or inclusion complexes of molecular rotors and motors in a solid matrix (4).

The group have also been very active in developing new methods of NMR crystallography, i.e. methods that combine experimental solid-state NMR data with theoretical calculations for gaining new insights into the structure and dynamics of solids and the intermolecular interactions in molecular crystals (5). For example, they have demonstrated that nuclear quantum effects in molecular crystals can be studied by SS-NMR spectroscopy and that path-integral molecular dynamics simulations are a suitable method for the incorporation of these effects into theoretical calculations.

They have been using computational chemistry also in other studies. For example, the group have been developing new methods for precise calculations of NMR parameters including anharmonic vibration corrections, the effects of dynamics, and solvation. Theoretical computations in combination with NMR experiments are also an indispensable tool in investigations of reaction mechanisms (6).

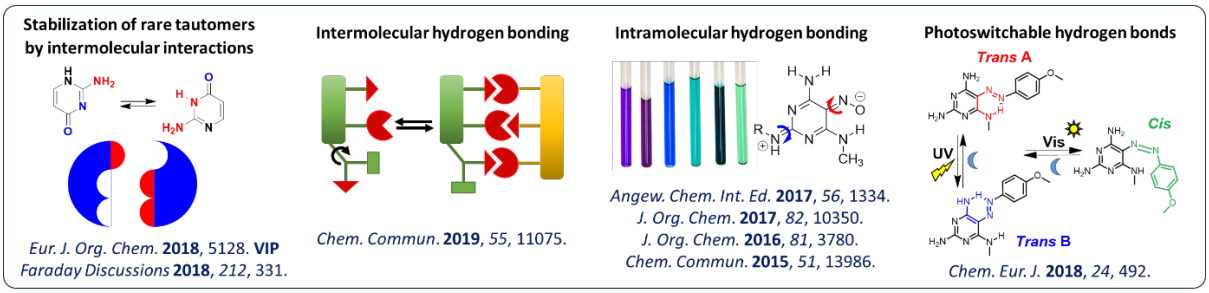


Research in NMR Spectroscopy: Material Research

The group have a longstanding research interest in non-covalent interactions, particularly hydrogen bonding (both intra- and inter-molecular). They have, for example, investigated the relationship between resonance stabilization of hydrogen bonds and nuclear quantum effects, such as proton delocalization and tunnelling (7). They also developed a new methodology for the determination of free energy changes associated with the formation of hydrogen-bonded pairs of modified nucleobases (8).

The group have also investigated proton transfers between hydrogen-bonded bases of nucleic acids and demonstrated that experimental NMR spectroscopy combined with advanced theoretical simulations provide an excellent tool for the investigations of proton transfers. Furthermore, they demonstrated that intermolecular interactions can stabilize non-canonical tautomers of nucleobases, which supports the hypothesis of the involvement of rare nucleobase tautomers in the catalytic function of RNA enzymes (9, highlighted as Very Important Paper).

The NMR Spectroscopy Group also discovered a new class of azocompounds with unique photoswitchable intramolecular hydrogen bonds with two hydrogen bond donors. Using suitable substituents, orthogonal photoswitching could be achieved. For example, whereas UV irradiation caused switching between the two rotamers of the *trans* isomer of a compound, visible light enabled to obtain the *cis* photoisomer (10).



Research in NMR Spectroscopy: Revealing Fundamental Interactions

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2. Čechová, L.; Kind, J.; Dračínský, M.; Filo, J., Janeba, Z.; Thiele, C. M.; Cigáň, M.; Procházková, E. Photoswitching Behavior of 5-Phenylazopyrimidines: In Situ Irradiation NMR and Optical Spectroscopy Combined with Theoretical Methods. *J. Org. Chem.* **2018**, 83, 5986–5998.

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4. Procházková, E.; Cao, C.; Rawal, A.; Dračínský, M.; Bhattacharyya, S.; Císařová, I.; Hook, J.; Stenzel, M. Polymorphic Transformation of Drugs Induced by Glycopolymeric Vesicles Designed for Anticancer Therapy Probed by Solid-State NMR Spectroscopy. *ACS Appl. Mater. Interfaces* **2019**, 11, 28278–28288.

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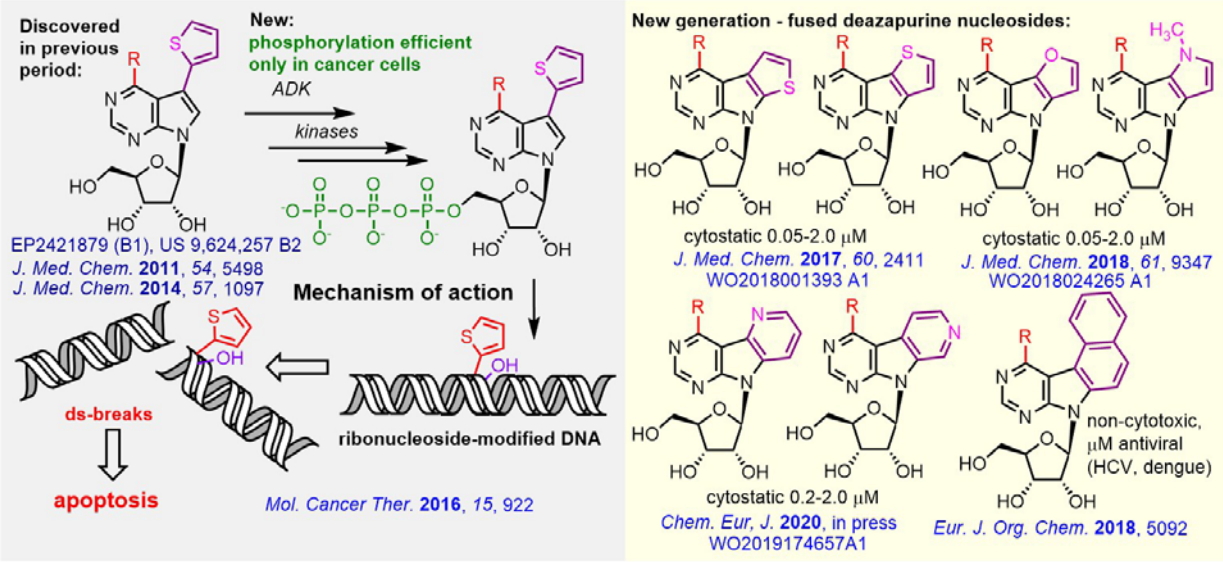
8. Štoček, J. R.; Bártová, K.; Čechová, L.; Šála, M.; Socha, O.; Janeba, Z.; Dračínský, M. Determination of nucleobase-pairing free energies from rotamer equilibria of 2-(methylamino)pyrimidines. *Chem. Commun*. **2019**, *55*, 11075–11078.
9. Pohl, R.; Socha, O.; Šála, M.; Rejman, D.; Dračínský, M. The Control of the Tautomeric Equilibrium of Isocytosine by Intermolecular Interactions. *Eur. J. Org. Chem.* **2018**, 5128–5135.
10. Procházková, E.; Čechová, L.; Kind, J.; Janeba, Z.; Thiele, C. M.; Dračínský, M. Photoswitchable Intramolecular Hydrogen Bonds in 5-Phenylazopyrimidines Revealed By In Situ Irradiation NMR Spectroscopy. *Chem. Eur. J.* **2018**, *24*, 492–498.

A complete list of publications can be found in ASEP.

Research activity and characterisation of the main scientific results

1. Medicinal Chemistry

1.1. The Hocek group has continued the development and study of structure-activity relationship of the series of highly cytostatic 7-substituted 7-deazapurine ribonucleosides discovered during the previous period of evaluation. The study of metabolism and mechanism of action revealed a completely unexpected and unprecedented mode of action consisting in specific activation of the ribonucleoside to triphosphate and its incorporation not only to RNA (where it caused inhibition of translation on ribosome) but also to genomic DNA (where the presence of the ribonucleoside caused double-strand breaks and apoptosis) (1,2). The series has then been expanded to a new class of analogues containing another aromatic or heterocyclic ring fused to the deazapurine moiety. Also in this second generation of fused nucleosides, highly potent derivatives with nanomolar cytostatic activity and good selectivity toward cancer cell lines have been discovered (3,4). Other types of extended fused nucleosides were non-cytotoxic but still showed interesting antiviral activities against certain RNA viruses and some others were fluorescent and were used for labelling of DNA or RNA. Selected most active compounds are undergoing preclinical ADMETox and *in vivo* activity screening in order to identify suitable candidates for further development toward antitumor agents. The whole programme is performed in close collaboration with the group of Dr. M. Hajduch (IMTM, Palacky University Olomouc), where the Hocek group is responsible for the design, synthesis and biochemical profiling, whereas the Hajduch group performs the cytostatic activity screening, additional biological assays and *in vivo* testing. Another on-going medchem project in the Hocek group deals with the design and synthesis of novel selective heterocyclic kinase inhibitors but at this stage we cannot reveal the structures due to pending patent applications. The group is also working on synthetic methodology and recently developed a conceptually novel method for glycosylation of heterocycles with in situ generated sugar-epoxides (5).



Selected key publications: (1) Perlíková, P.; Rylová, G.; Nauš, P.; Elbert, T.; Tloušťová, E.; Bourderioux, A.; Poštová Slavětínská, L.; Motyka, K.; Doležal, D.; Znojek, P.; Nová, A.; Harvanová, M.; Džubák, P.; Šiller, M.; Hlaváč, J.; Hajdúch, M.; Hocek, M. *Mol. Cancer Ther.* **2016**, *15*, 922-937. (2) Perlíková, P.; Hocek, M. *Med. Res. Rev.* **2017**, *37*, 1429-1460. (3) Tichý, M.; Smoleň, S.; Tloušťová, E.; Pohl, R.; Oždian, T.; Hejtmánková, K.; Lišková, B.; Gurská, S.; Džubák, P.; Hajdúch, M.; Hocek, M. *J. Med. Chem.* **2017**, *60*, 2411-2424. (4) Tokarenko, A.; Lišková, B.; Smoleň, S.; Táborská, N.; Tichý, M.; Gurská, S.; Perlíková, P.;

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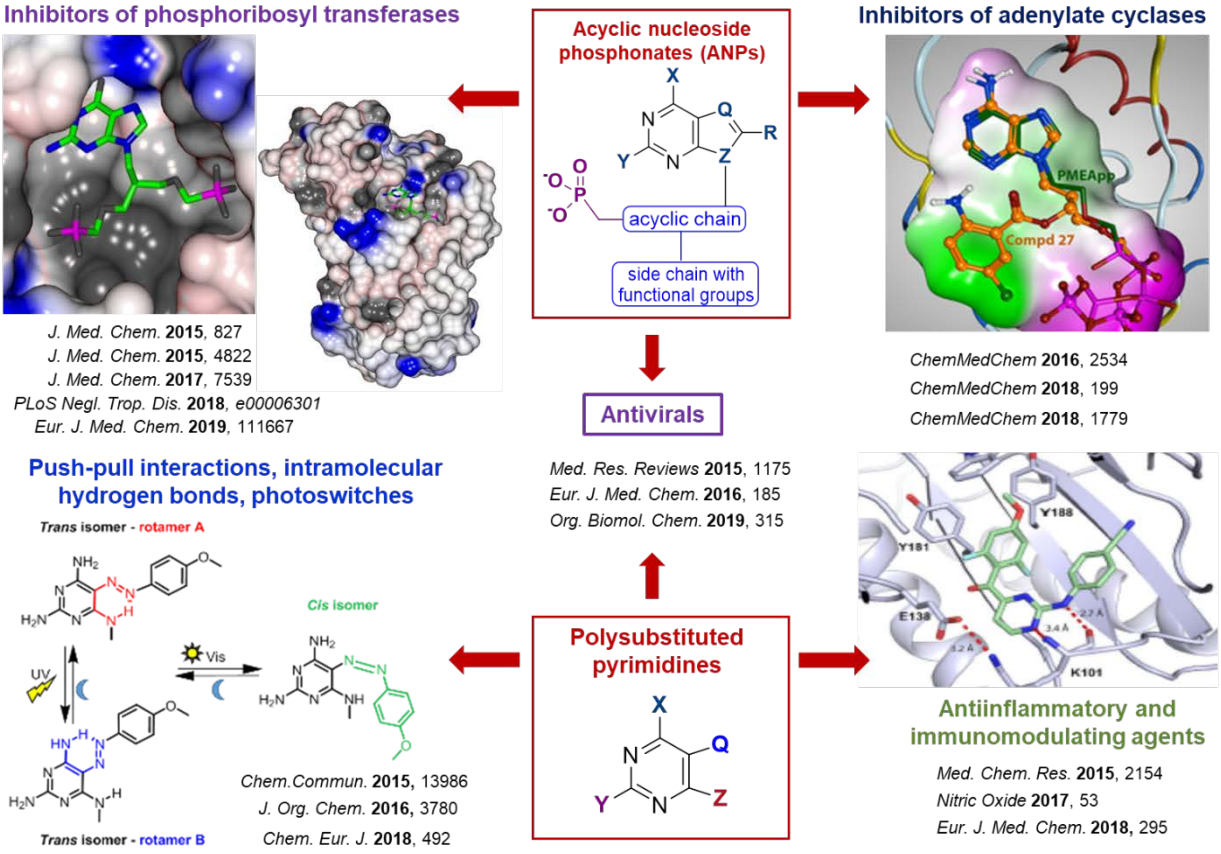
1.2. The Janeba group put an effort into two major research areas: 1) preparation of novel acyclic nucleoside phosphonates (ANPs, incl. bisphosphonates); 2) synthesis of polysubstituted pyrimidines. Novel ANPs and aza-ANPs were designed and prepared as potent inhibitors of hypoxanthine-guanine-(xanthine) phosphoribosyltransferases (HG(X)PRTs) from *Plasmodium falciparum* and/or *P. vivax* (1,2). Prodrugs of these nucleotide analogues exhibited antimalarial activity with IC_{50} values of 0.8 – 6.0 μM. Selected ANPs inhibited also *Mycobacterium tuberculosis (Mtb)* HGPRT and *Trypanosoma brucei (Tbr)* HG(X)PRTs. Their prodrugs were active against *Mtb* and *Tbr* in cell-based assays (3,4). Another novel class of ANPs was designed as potent inhibitors of human purine nucleoside phosphorylase (PNP) and these compounds (and their prodrugs) are included in preclinical studies for their potent anti-leukemic properties (patent application is pending). Some of these compounds are also able to inhibit PNP from *Mtb*. Novel derivatives of adefovir (PMEA) were evaluated as potent inhibitors of bacterial adenylate cyclases. Antiviral properties of selected ANPs and substituted pyrimidines (NNRTIs) were studied.
Large series of polysubstituted pyrimidines was evaluated for their anti-inflammatory properties, where inhibition of PGE_2 and/or NO production is the key mode of action. Another project deals with synthesis and evaluation of physicochemical properties of substituted 5-nitroso- (5) and 5-phenylazopyrimidines (6). Prepared polysubstituted pyrimidines were studied for their strong intramolecular hydrogen bonds - IMHBs (5), push-pull interactions (5) or their interesting photoswitchable IMHBs (6). In case of certain 5-phenylazopyrimidines with appropriate substituents, orthogonal photoswitching was achieved.

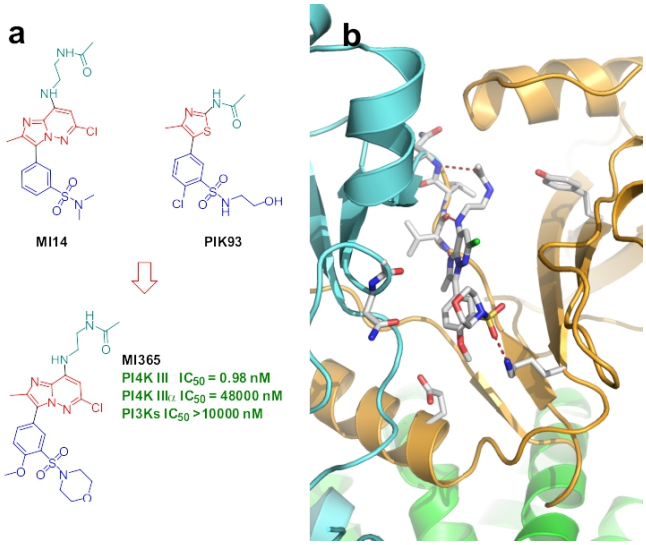


Selected key publications: (1) Keough, D. T.; Hocková, D.; Janeba, Z.; Wang, T.-H.; Naesens, L.; Edstein, M. D.; Chavchich, M.; Guddat, L. W. *J. Med. Chem.* **2015**, *58*, 827–846. (2) Špaček, P.; Keough, D. T.; Chavchich, M.; Dračínský, M.; Janeba, Z.; Naesens, L.; Edstein, M. D.; Guddat, L. W.; Hocková, D. *J. Med. Chem.* **2017**, *60*, 7539–7554. (3) Eng, W. S.; Hocková, D.; Špaček, P.; Janeba, Z.; West, N. P.; Woods, K.; Naesens, L.; Keough, D.; Guddat, L. *J. Med. Chem.* **2015**, *58*, 4822–4838. (4) Doleželová, E.; Teran, D.; Gahura, O.; Kotrbová, Z.; Procházková, M.; Keough, D.; Špaček, P.; Hocková, D.; Guddat, L.; Zíková, A. *PLoS Negl. Trop. Dis.* **2018**, *12(2)*: e0006301. (5) Procházková, E.; Čechová, L.; Tarábek, J.; Janeba, Z.; Dračínský, M. *J. Org. Chem.* **2016**, *81*, 3780–3789. (6) Procházková, E.; Čechová, L.; Kind, J.; Janeba, Z.; Thiele, C. M.; Dračínský, M. *Chem Eur. J.* **2018**, *24*, 492–498.

1.3. The Nencka group has recently achieved significant successes in the following areas:...

1) Structure-based design of selective phosphatidylinositol 4-kinase III□□inhibitors as potential antiviral agents and tools for chemical biology

Phosphatidylinositol 4-kinase III□□(PI4KIII□) serves as an important host factor for replication of various +RNA viruses. Based on results from both crystallographic and docking studies, we designed novel PI4KIII□ inhibitors and identified compounds with outstanding inhibitory activity and selectivity for this isoform in comparison with other protein and lipid kinases. To the best of our knowledge, our compound MI365 possesses the highest inhibitory activity reported for PI4KIII□ (see the figure).[1] Selected compounds from this series are currently being evaluated for ADMET properties *in vitro* and *in vivo* (crystallographic analysis was done in the Boura lab).



Within the kinase-oriented project, the group have also focused on novel synthetic approaches toward bicyclic heterocycles (numerous kinase inhibitors contain this type of central core in their structures). One of the most significant results of this research was the discovery of a new synthetic route to pyrimido[4,5-d]pyridazines directly from pyrimidines and tetrazines under basic conditions.[2]

2) Nucleosides, nucleotides and cyclic dinucleotides as antiviral agents

Within this field, the group have recently achieved two major results. First, arthropod-borne flaviviruses have been of interest to the group for several years, which gave it a significant advantage during the recent outbreak of the Zika virus (ZIKV). The group is a part of a team that discovered the first nucleoside derivatives active against this dangerous pathogen.[3] The team has also prepared triphosphate derivatives of selected nucleosides and proved that they effectively inhibit RNA-dependent RNA polymerase (RdRp). The team was also the first academic facility to describe an effective ZIKV polymerase assay.[4] In addition, the group have recently prepared a model ZIKV RdRp, which should serve as a functional tool for development of novel nucleoside/tide analogues for treatment of flavivirus infections. (Biochemical and virology experiments were done in the Boura group at the IOCB and the Ruzek group at the Biology Center CAS).

Second, we have recently identified several cyclic dinucleotides that are highly potent agonists of STING (stimulator of interferon genes), which could be useful in antiviral and anticancer therapy. The licensing of these compounds to major pharmaceutical company is currently under way (in collaboration with the Birkus lab at the IOCB).

3) Neutral sphinghomyelinase 2 inhibitors as novel antiviral compounds and prospective treatments for neurodegenerative diseases

Neutral sphinghomyelinase 2 (nSMase2) plays an important role in exosome release. This is a cellular process strongly implicated in various neurodegenerative diseases such as Alzheimer disease, but also in the pathogenesis of several viruses including HIV. Optimization of the HTS hit resulted in compound MS882, a potent, orally available, brain penetrable nSMase2 inhibitor, which inhibits exosome release both *in vitro* and *in vivo*. We have recently filed a patent application on the whole series of nSMAse2 inhibitors and published this research results in BJP[5] (in collaboration with the Slusher group at Johns Hopkins University).

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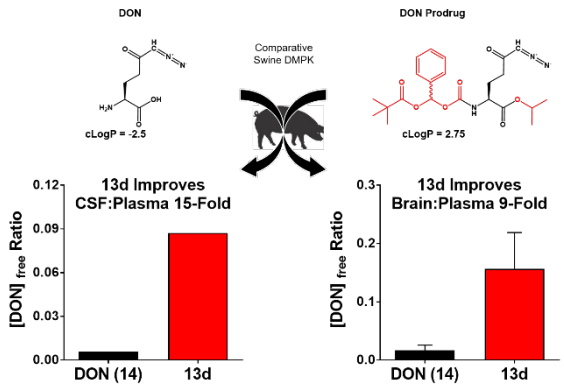
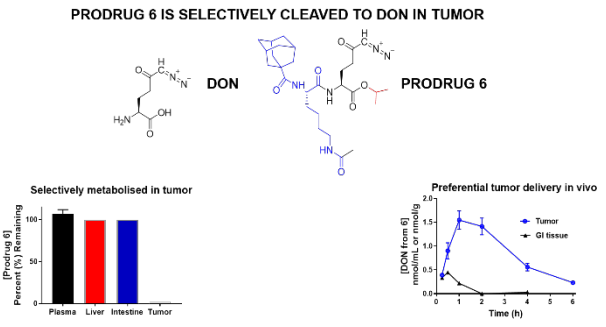
1.4. The Majer group (Drug Discovery) main focus is design and synthesis of potential therapeutics for cancer. The most advanced project is based on prodrugs of a glutamine antimetabolite 6-diazo-5-oxo-L-norleucine (DON). The naturally occurring DON is a covalent irreversible inhibitor of most glutamine utilizing enzymes and has a strong anti-tumor activity but also significant gastrointestinal toxicity. Prodrugs of DON made by Majer group deliver the active agent predominantly into tumor cells protecting thus the gastrointestinal tract (1, 2). Compounds were tested in several animal models at Johns Hopkins University in Baltimore, MD (USA) and the technology (3) was licensed to Dracen Pharmaceuticals Inc. The preclinical testing was successfully concluded in 4/2020 and the candidate compound entered the phase 1 clinical trial. The first patients were dosed in 8/2020. New class of DON prodrugs with improved solubility selectivity and efflux profile was recently developed (4).



J. Med. Chem. **2017, 60, 7186–7198**

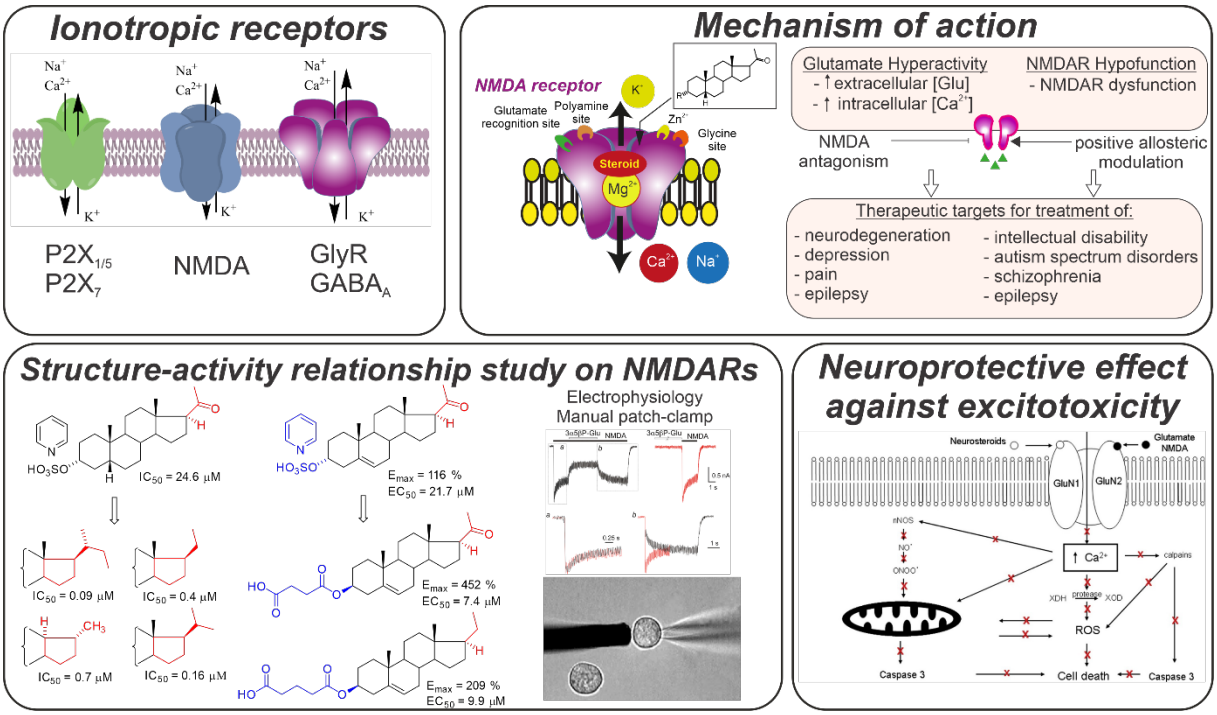


J. Med. Chem. **2019, 62, 3524–3538**

The second most advanced project is focused on inhibitors of Glutamate Carboxypeptidase II (GCPII) a.k.a. Prostate Specific Membrane Antigen (PSMA) and has two arms. The first one pursues development of prodrugs of 2-phosphonomethyl-pentanedioic acid (2-PMPA), a potent inhibitor of GCPII, as kidney protecting agent for prostate cancer radiotherapy (5). The second one is based on 2-PMPA conjugates with deoxycholic acid to be used as therapeutics for colitis and Crohn's disease. The technology of the first arm has recently been licensed by PSMA Therapeutics (6).

Selected key publications: (1) Rais, R., Jančařík, A., Tenora L., Nedelcovych, M., Alt, J., Englert, J., Rojas, C., Le, A., Elgogary, A., Tan, J., Monincová, L., Pate, K., Adams, R., Ferraris, D., Powell, J., Majer, P., Slusher, B.S. *J. Med. Chem.* **2016,** *59*, 8621-8633**.** (2) Nedelcovych, M.T.; Tenora, L.; Kim, B.-H.; Kelschenbach, J.; Chao, W.; Hadas, E.; Jančařík, A.; Prchalová, E.; Zimmermann, S.C.; Dash, R.P.; Gadiano, A.J.; Garrett, C.; Furtmüller, G.; Oh, B.; Brandacher, G.; Alt, J.; Majer, P.; Volsky, D.J.; Rais, R.; Slusher, B.S. *J. Med. Chem.* **2017**, *60*, 7186–7198. (3) Majer, P.; Jančařík, A.; Tenora, L.; Rais, R.; Slusher, B.S. Prodrugs of glutamine analogs, **US 10336778** (4) Tenora, L.; Alt, J.; Dash, R.P.; Gadiano, A.J.; Novotná, K.; Veeravalli, V.; Lam, J.; Kirkpatrick, Q.R.; Lemberg, K.M.; Majer, P.; Rais, R.; Slusher, B.S. *J. Med. Chem.* **2019**, *62*, 3524–3538. (5) Majer, P.; Jančařík, A.; Krečmerová, M.; Tichý, T.; Tenora, L.; Wozniak, K.; Wu, Y.; Pommier, E.; Ferraris, D.; Rais, R.; Slusher, B.S. *J. Med. Chem.* **2016**, *59*, 2810–2819. (6) Majer, P.; Jančařík, A.; Krečmerová, M.; Tichý, T.; Rais, R.; Slusher, B.S. Prodrugs of prostate specific membrane antigen (PSMA) inhibitor, **US 9988407.**

1.5. The Kudová group has continued in the development and study of structure-activity relationship of neurosteroids as allosteric inhibitors (1-3) of *N*-methyl-*D*-aspartate receptors (NMDARs) as pathological overactivation of NMDARs leads to excitotoxicity, a specific form of neuronal cell death that is thought to underlie various forms of neurodegeneration, such as Alzheimer's disease, ischemia, or traumatic brain injury. Additionally, we have extended our research interest to positive allosteric modulators (4,5) of NMDARs. The increased activity of NMDARs may provide a therapeutic aid for patients suffering from neuropsychiatric disorders, where NMDARs hypofunction is thought to be involved, such as intellectual disability, autism spectrum disorder, or schizophrenia (6). Novel molecules are tested for their modulatory activity by patch-clamp technique on transfected HEK293 cells in collaboration with the group of prof. L. Vyklicky (IPhys, Czech Academy of Sciences). Selected active compounds are undergoing preclinical ADMETox and screening and testing in collaboration with dr. Mertlikova-Kaiserova (IOCB) in order to identify suitable candidates for further development. Novel methods and assays have been recently developed and results published (7,8).
As neurosteroids produce rapid effects on neuronal excitability and synaptic function that involve direct or indirect modulation of various neurotransmitter-gated ion channels, we have recently extended our scientific portfolio to modulation of *(i)* glycine receptors as those are the major inhibitory receptors in the spinal cord and the brain stem (9); *(ii)* ATP-gated purinergic P2X receptors due to their involvement in pain, inflammation, osteoporosis, and multiple sclerosis (10). Finally, extensive drug development of novel compounds has been currently ongoing on neurosteroids as potential therapeutics for the treatment of neuropathic pain (in collaboration with Dr. Paleček Dr. Jakubík, IPhys, Czech Academy of Sciences) and of epilepsy ((in collaboration with Dr. Kubová, IPhys, Czech Academy of Sciences). Novel structures, mode of action and therapeutic targets have been protected by national and international patent applications and filled patents (2,11).



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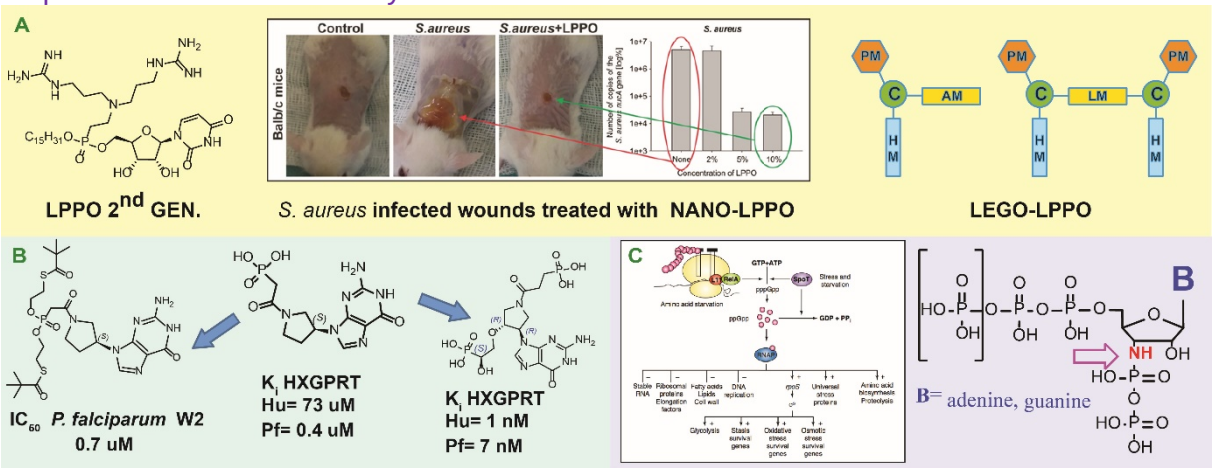
1.6. The Rejman group is focused to development of novel strategies in combating microbial infections. We work on three distinct projects. The first one (**A**) deals with design, synthesis and properties of antimicrobial compounds selectively disrupting bacterial membrane called **lipophosphonoxins** (**LPPO**). Synthesis and properties of second generation **LPPO** has been published (1) and patented (WO2017186200A1). We evaluated use of LPPO 2nd gen as an additive to bone cement (2) as well as in combination with nanomaterial-based dressing (NANO-LPPO) for treatment of topical infections (manuscript in preparation). Next, we performed next round of SAR experiments resulting in modular platform for semi-rational design of membrane active antimicrobials termed **LEGO-LPPO** (patent submitted 2019, MS in preparation). **LEGO-LPPO** consist of connector module (**C**) polar module (**PM**), hydrophobic module (**HM**), linker module (**LM**) or auxiliary module (**AM**). It is a multidisciplinary collaborative project where several institutions participate, namely Palacký University Olomouc (M. Kolář) and Institute of Microbiology CAS (Krásný). The Rejman group is responsible for coordination, design and synthesis.

In the second project (**B**), we design and synthesize nucleotide analogs based on pyrrolidine scaffold as potential hypoxanthine-xanthine-guanine-phosphoribosyltransferase (HXGPRT) inhibitors. Prodrugs of these inhibitors are intended to be used as antimalarial and/or antituberculosis agents (3, 4). This project is carried out in collaboration with the University of Queensland (L. Guddat and D. Keaugh). The Rejman group is responsible for design and synthesis.

Under stressful conditions, bacterial RelA-SpoT Homolog (RSH) enzymes synthesize the alarmone (p)ppGpp, a nucleotide second messenger. (p)ppGpp rewires bacterial transcription and metabolism to cope with stress, and, at high concentrations, inhibits the process of protein

synthesis and bacterial growth to save and redirect resources until conditions improve. In the third project (**C**), the main goal is to elucidate this regulatory pathway as a new target for therapy of infectious diseases. Here the Rejman group is responsible for developing synthetic strategies for molecular tools (e.g. (p)ppApp, nonhydrolyzable ppGpp analogue (p)ppG$_N$pp) (5), and also for design and synthesis of inhibitors of involved enzymes (6). University of Umea (V. Hauryliuk) is responsible for biochemistry, Université Libre de Bruxelles (A. Garcia-Pino) is responsible for structural analyses.



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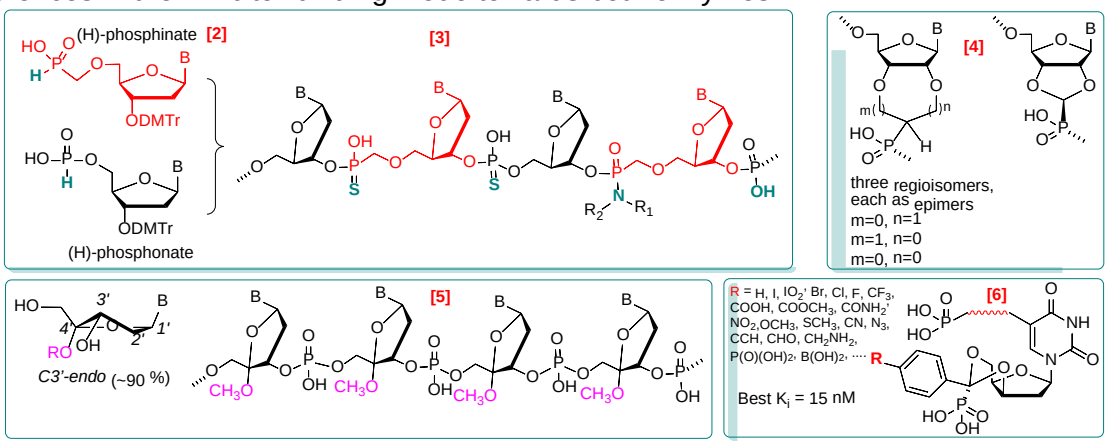
(3) Keough D. T. et al., Design of Plasmodium vivax Hypoxanthine-Guanine Phosphoribosyltransferase Inhibitors as Potential Antimalarial Therapeutics. *ACS Chem. Biol.* **2018**, *13*, 82–90.
(4) Eng W. S. et al., Pyrrolidine nucleoside bisphosphonates as antituberculosis agents targeting hypoxanthine-guanine phosphoribosyltransferase. *Eur. J. Med. Chem.* **2018**, *159*, 10-22.
(5) Jimmy, S. et al., A widespread toxin−antitoxin system exploiting growth control via alarmone signaling. *Proc. Natl. Acad. Sci. USA* **2020**, *117*, 10500-10510.
(6) Beljantseva, J. et al., Molecular mutagenesis of ppGpp: turning a RelA activator into an inhibitor. *Sci. Rep.* **2017**, *7*, 41839.

1.7. The Rosenberg group. Our fundamental results on increased RNase H cleavage rate of RNA strand of hybrid duplexes containing a phosphonate oligodeoxynucleotide (ON) strand (1) has led to collaboration with *Alios (*now *Janssen) BioPharma Ltd*. (*Johnson & Johnson*) oriented to the search for phosphonate-based (i) CpG ONs as TLR-9 agonists and (ii) antisense ONs working by RNase H mechanism as potential drugs for treatment of viral and malignant diseases. Biological relevance of phosphonate ONs has prompted us to elaborate single-chemistry method for the introduction of both natural and phosphonate nucleotide units. The combination of phosphoramidite and advanced phosphotriester chemistries used so far as the only alternative could be successfully substituted by H-phosphonate chemistry due to development of straightforward synthesis of the monomers – the nucleoside-*O*-methyl-(*H*)-phosphinates (2) related to classic nucleoside (*H*)-phosphonates. Both types of monomers can be combined in the oligonucleotide chains under formation of (H)-phosphinate (3'C-O-P(O)(H)-C-O-C5') and (H)-phosphonate (3'C-O-P(O)(H)-O-C5') internucleotide linkages which can be oxidized, sulfurized, and amidated to -P(O)(OH)-, -P(S)(OH)-, and -P(NR'R'')(O)- types of bonds (3), respectively. This approach has allowed us to increase diversity of phosphonate ONs for biological studies. The results on thermal stability of heteroduplexes of 1,3-dioxolane- and 1,4-dioxane-locked phosphonate ONs bearing 2',3'-*O*-phosphonomethylidene and 2',3'-*O*-phosphonoethylidene internucleotide linkages with target RNA provided relatively large extent of Tm values with best one +5.2°C per modification(4) – the correlation between the experimental T_m values and the number of nucleobase pairs measured as distances between atoms 1-*N* (A,G) and 3-*N* (U,C)] that remained stable in ten independent MD runs

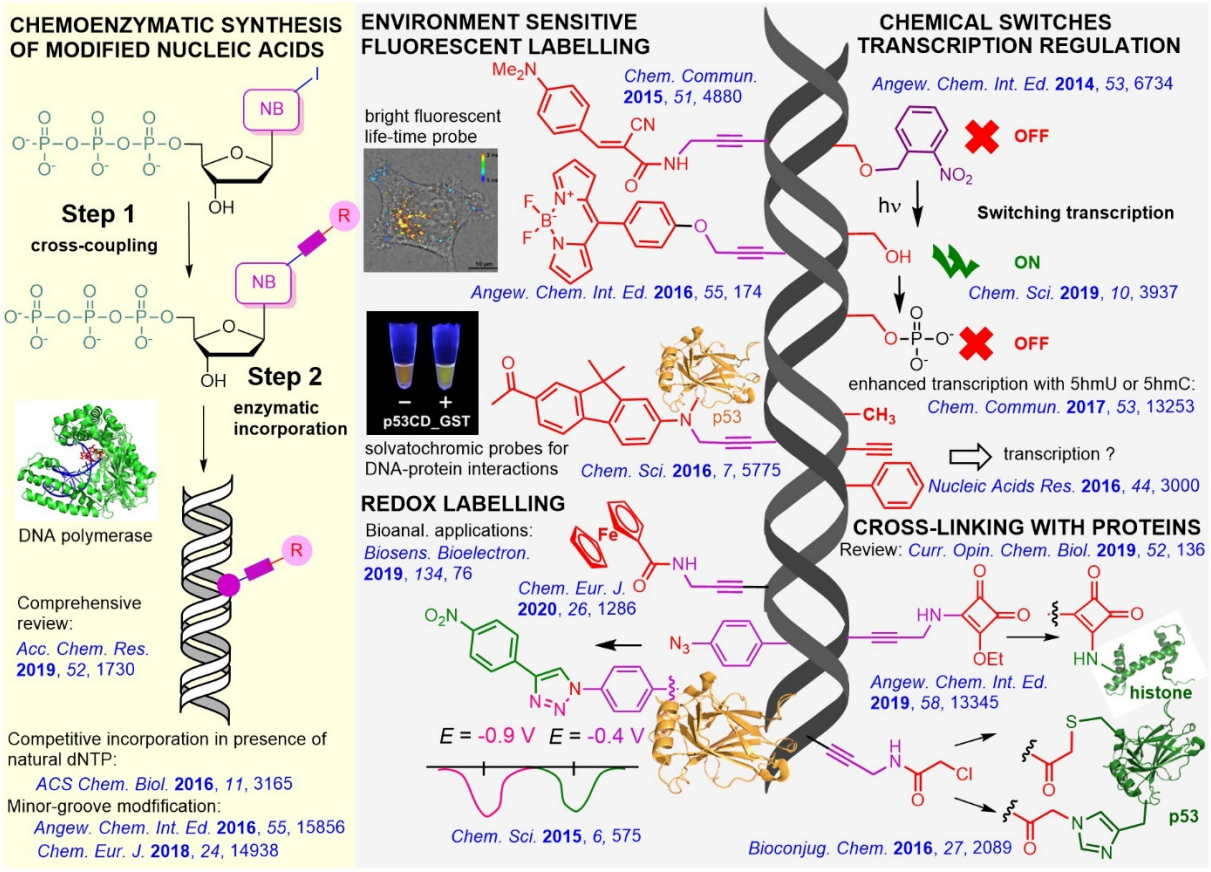
lasting for 120 ns was found. Novel phosphodiester 4'-alkoxyoligodeoxynucleotides(5) were synthesized and evaluated as a new RNA-type (>80% of *C3'-endo* sugar pucker) of compounds exhibiting superior chemical stability of nucleosidic bond and nuclease resistance as well as strong hybridization properties with obvious tendency for RNA-selective hybridization, suggesting a potential application in antisense technologies, *e.g.*, as flanks in gapmers. In a close collaboration with the *Group of Structural Biology of IOCB* we have discovered a most potent group of nucleoside bis-phosphonate inhibitor(6) so far known, mimicking both substrate (dTMP) and phosphate anion, and achieved 100-fold increases in the inhibitory potency towards pyrimidine specific cytosolic and mitochondrial deoxynucleotidases, respectively. Crystal structures of both complexes showed major differences in the inhibitor binding mode towards both enzymes.



Selected key publications: (1) Sipova, H. et al. 5'-O-Methylphosphonate nucleic acids-new modified DNAs that increase the Escherichia coli RNase H cleavage rate of hybrid duplexes. *Nucl. Acids Res.* **2014**, *42*, 5378-5389. (2) Kostov, O. et al. Solid-Phase Synthesis of Phosphorothioate/Phosphonthioate and Phosphoramidate and/or Phosphonamidate Oligonucleotides. *Molecules* **2019**, 24, 1872. (3) Kostov, O.; Páv, O. et al. 4-Toluenesulfonyloxymethyl-(H)-phosphinate: A Reagent for the Introduction of O- and S-Methyl-(H)-phosphinate Moieties. *Org. Lett.* **2016**, 18, 2704-2707; Curr. Protoc. Nucleic Acid Chem. **2017**, 70: 4.76.1-4.76.22. (4) Páv, O. et al. Tuning the hybridization properties of modified oligonucleotides: from flexible to conformationally constrained phosphonate internucleotide linkages. *Org. Biomol. Chem.* **2017**, *15*, 701-707. (5) Petrová, M. et al. Straightforward Synthesis of Purine 4'-Alkoxy-2'-deoxynucleosides: First Report of Mixed Purine-Pyrimidine 4'-Alkoxyoligodeoxynucleotides as New RNA Mimics. *Org. Lett.* **2015**, *17*, 3426-3429; *Curr. Protoc. Nucleic Acid Chem.* **2016**, 66: 1.38.1-1.38.27.; (6) Pachl, P. et al. Structure-based design of a bisphosphonate 5 '(3')-deoxyribonucleotidase inhibitor. *MedChemComm*. **2015**, *6*, 1635-1638.

2. Bioorganic chemistry

2.1. The Hocek group has continued their development of chemoenzymatic methodology for synthesis of modified nucleic acids through polymerase incorporation of modified nucleotides (1). Different methods have been developed for synthesis of short or long oligonucleotides, DNA or RNA and the resulting modified nucleic acids have been used in diverse applications. Redox or fluorescent labelling of nucleic acids was used for bioanalysis or imaging (collaboration with M. Hof, JHIPC in phospphysics or with M. Fojta IBP CAS or C. O'Sullivan, URV, in electrochemistry) (2). Reactive modifications were used for cross-linking with DNA-binding proteins (3). Modification of major groove of DNA and bioorthogonal reactions were used for chemical switching of transcription (collaboration with L. Krasny, IMB CAS, who did biological part of the project) (4). A new transporter for delivery of nucleoside triphosphates across the cell membrane was developed and used for imaging of genomic DNA in live cells (5).



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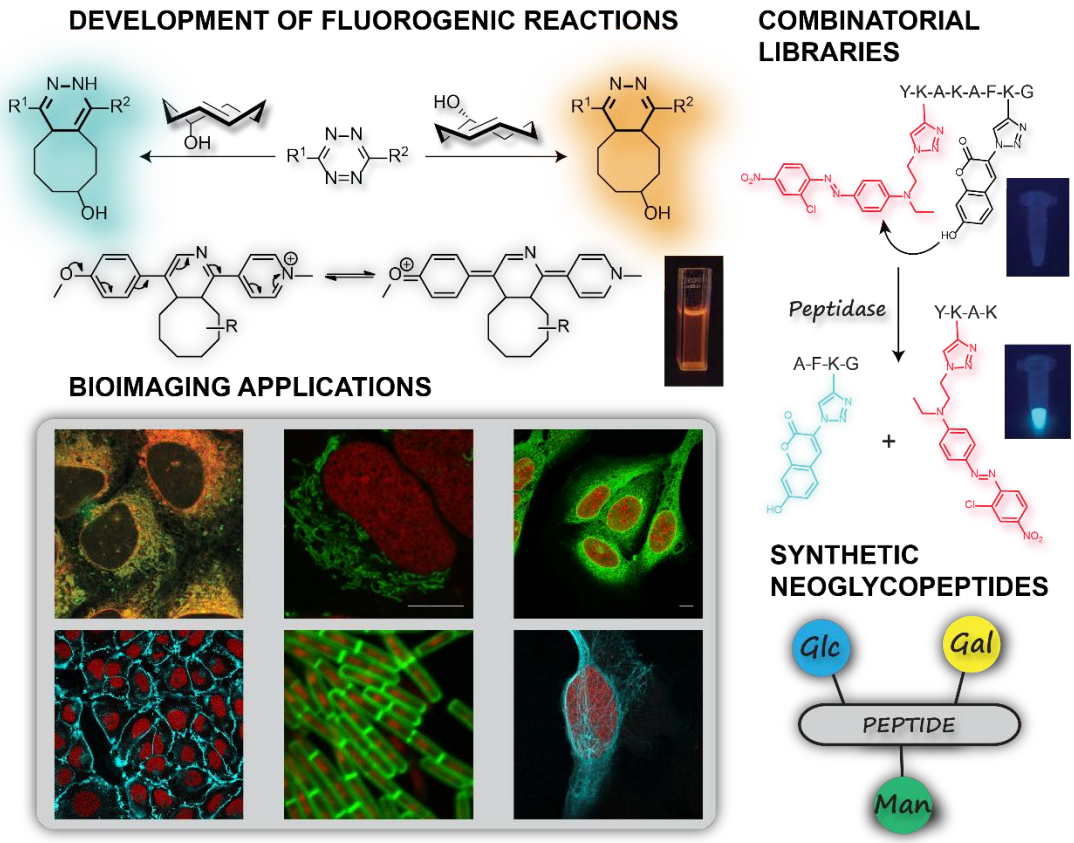
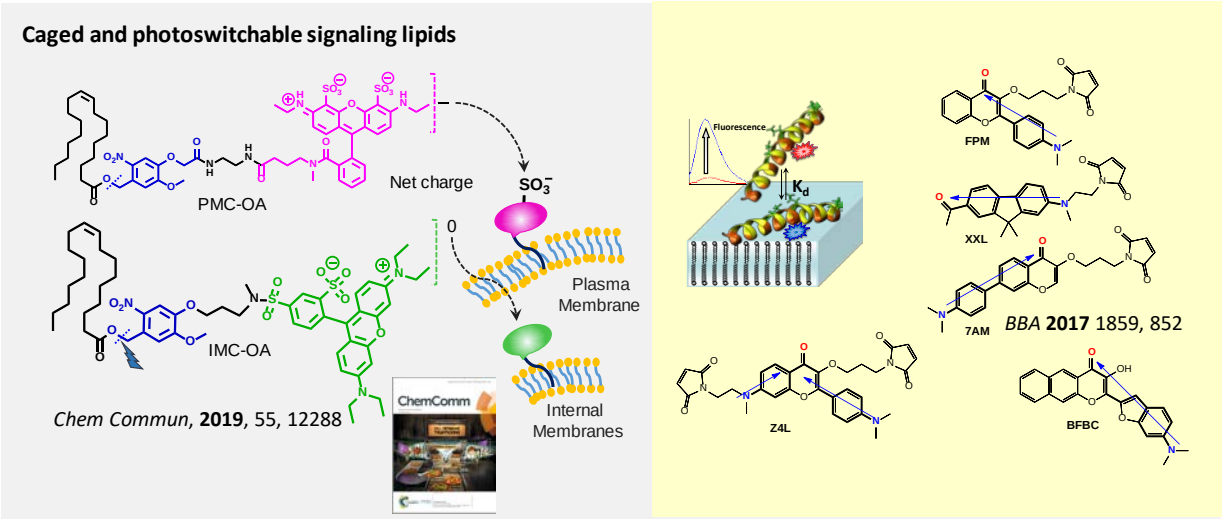
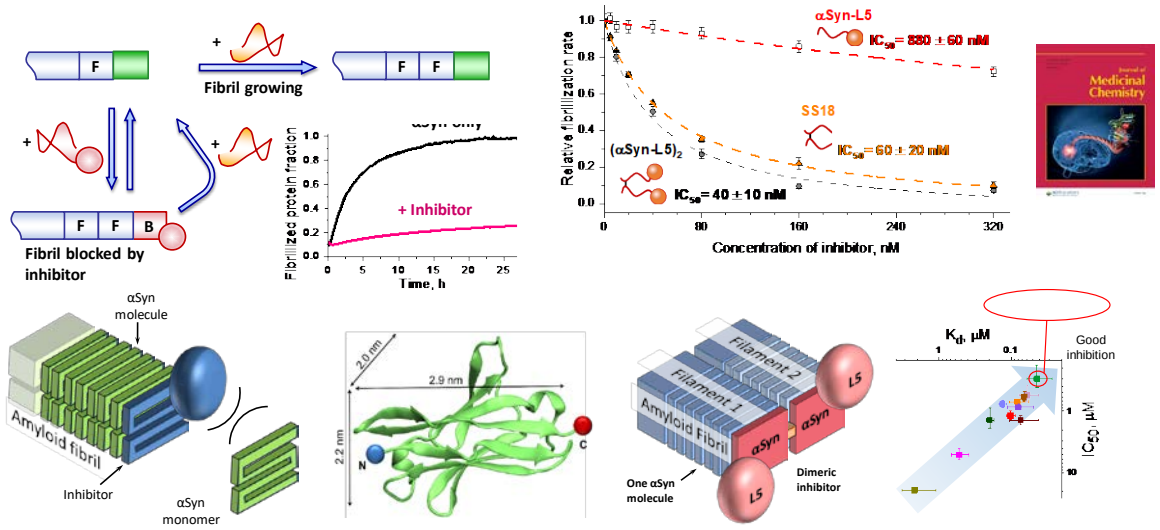
2.2 The Vrábel group developed a series of fluorogenic bioorthogonal reagents, which light-up in reaction with the complementary reacting partners. The reagents enable fast and biocompatible fluorescent labeling of biomolecules and small molecules directly in- and outside living cells with excellent signal-to-noise ratio. The group also developed an efficient strategy for combinatorial synthesis of neo-glycopeptides that are used for studies of protein-sugar interactions.



Selected key publications: (1) Vazquez, A.; Dzijak, R.; Dračínský, M.; Rampmaier. R.; Siegl, S. J.; Vrabel, M. *Angew. Chem. Int. Ed.* **2017**, *56*, 1334-1337. (2) Siegl, S.; Dzijak, R.; Vazquez, A.; Pohl, R.; Vrabel M. *Chem. Sci.* **2017**, *8*, 3593-3598. (3) Siegl, S. J.; Vázquez, A.; Dzijak, R.; Dračínský, M.; Galeta, J.; Rampmaier, R.; Klepetářová, B.; Vrabel, M. *Chem. Eur. J.* **2018**, *24*, 2426-2432. (4) Kovalová, A.; Pohl, R.; Vrabel, M. *Org. Biomol. Chem.* **2018**, *16*, 5960 -5964. (5) Siegl, S. J.; Galeta, J.; Dzijak, R.; Vázquez, A.; Del-Río-Villanueva, M.; Dračínský, M.; Vrabel, M. *ChemBioChem* **2019**, *20*, 886-890.

2.3. The Yushchenko group worked on development of chemical tools for modulating and studying biological processes. They synthetized caged signalling lipids that can be selectively delivered to specific cellular compartments, activated by a UV light pulse, and induce Ca-signalling at desired moment (1, 2). The group also developed new environment-sensitive fluorescent dyes and labels to study interactions of amyloidogenic proteins (3, 4). They found a new approach to inhibit misfolding of Parkinson's disease related protein α-synuclein based on blocking ends of amyloid fibrils (5) and prepared series of inhibitors based on cross-linked(6) or fused proteins (7).





Angew Chem **2018**, 57, 5690

J Mol Biol **2020** 432, 967

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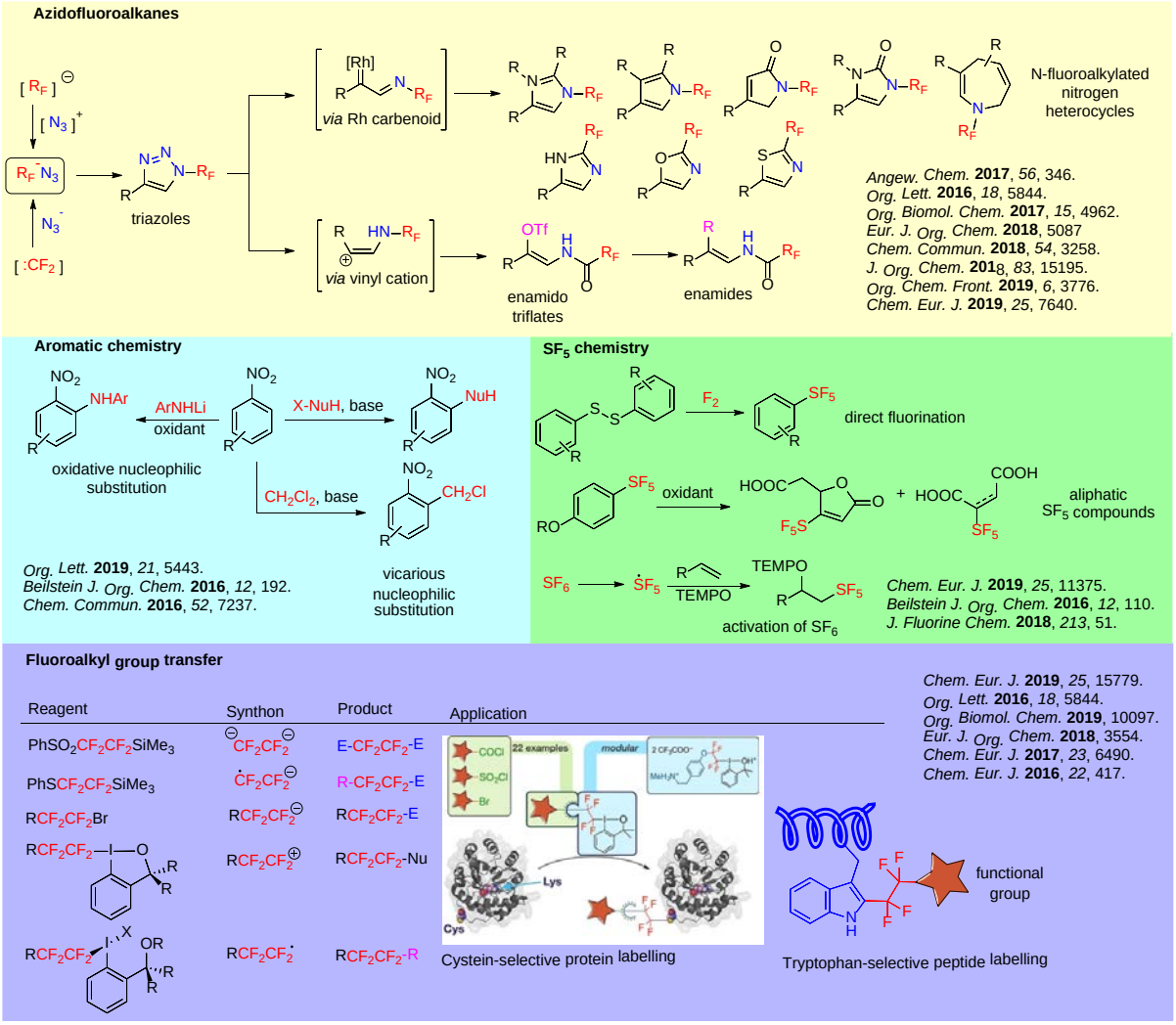
Research activity and characterisation of the main scientific results

1. Organic synthesis

1.1 The Beier group has continued research in several areas. In the area of fluorinated azidoalkanes, new synthetic pathway to azidotrifluoromethane was developed. Azidoperfluoroalkanes were introduced, azidodifluoromethane was synthetized via difluorocarbene and other previously unreported fluorinated azidoalkanes were prepared using original methodologies. Accessibility of these compounds allowed to study their stability and reactivity providing access to *N*-fluoroalkylated triazoles. These triazoles underwent Rh-catalyzed transannulations to new *N*-fluoroalkylated five- and seven-membered nitrogen heterocycles with potential applications in life-sciences. Reactions of triazoles with superacids afforded a new decomposition pathway via vinyl cation chemistry to synthetically useful enamido triflates and enamides. The topic of alfa fluorinated azidoalkanes was recently reviewed. In the area of aromatic chemistry, we have studied novel nucleophilic C-H substitution reactions either via oxidative nucleophilic substitution with nitrogen nucleophiles affording nitroanilines or via vicarious nucleophilic substitution with carbon nucleophiles to provide chloromethyl nitrobenzenes. In the area of SF_5 chemistry, we have studied novel access to aliphatic SF_5 compounds by oxidation of aromatics, direct fluorination of aromatic disulfides and thiols to arylsulfur pentafluorides and S-F activation of sulfur hexafluoride to afford SF_5 compounds. In the area of fluoroalkyl group transfer, we have introduced new reagents for nucleophilic, radical, and electrophilic CF_2CF_2 group transfer. The topic was reviewed. The reagents based on hypervalent iodine compounds were used in bioconjugation of cysteine residues in peptides and proteins for the attachment of various functional groups to these biomolecules. In the presence of suitable biocompatible reductant, the hypervalent iodine reagents also served as fluoroalkyl radical sources for selective bioconjugation of tryptophan residues in peptides and proteins. In the area of synthesis and modification of plant growth regulators, we have synthetized various bioactive seed germination inhibitors and promotors and studied structure-activity relationship and the mode of action of the bioactive butenolides.



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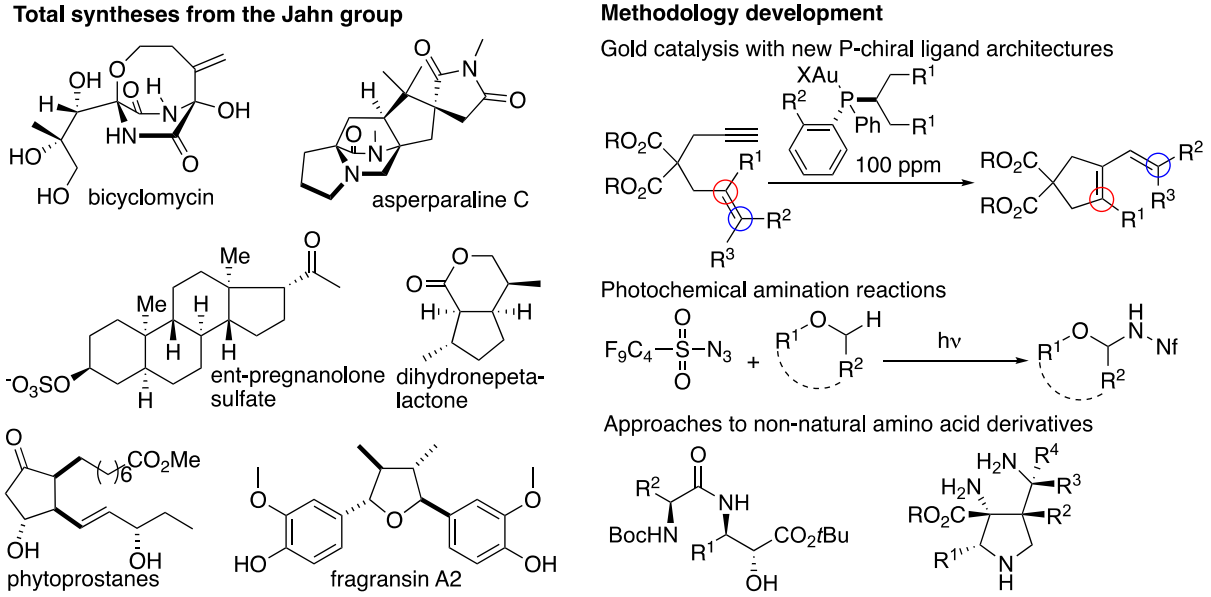
1.2 The Jahn group concentrated in the 2015–2019 period in the total synthesis area on synthetic approaches to bridged diketopiperazine alkaloids and related natural products. A unique approach to bicyclic diketopiperazines was developed, which culminated in a formal synthesis of the antibiotic bicyclomycin and in a 16-step total synthesis of *ent*-asperparaline C allowing the establishment of the absolute configuration of this complex pentacyclic alkaloid class (1,2). These results form the basis for currently ongoing total syntheses of pyrazinoquinazoline and diketopiperazine alkaloids. We accomplished a total synthesis of *ent*-steroids as molecular probes for the modulation of the NMDA receptor (3) and developed total syntheses of bicyclic terpenes, such as dihydronepetalactone. Long standing targets for total syntheses are autoxidatively formed cyclic lipid metabolites, where a unified approach to all major trioxygenated phytoprostanes was developed, which is the basis for identification of new metabolites and their signaling action in plants. A unified three-step approach to major

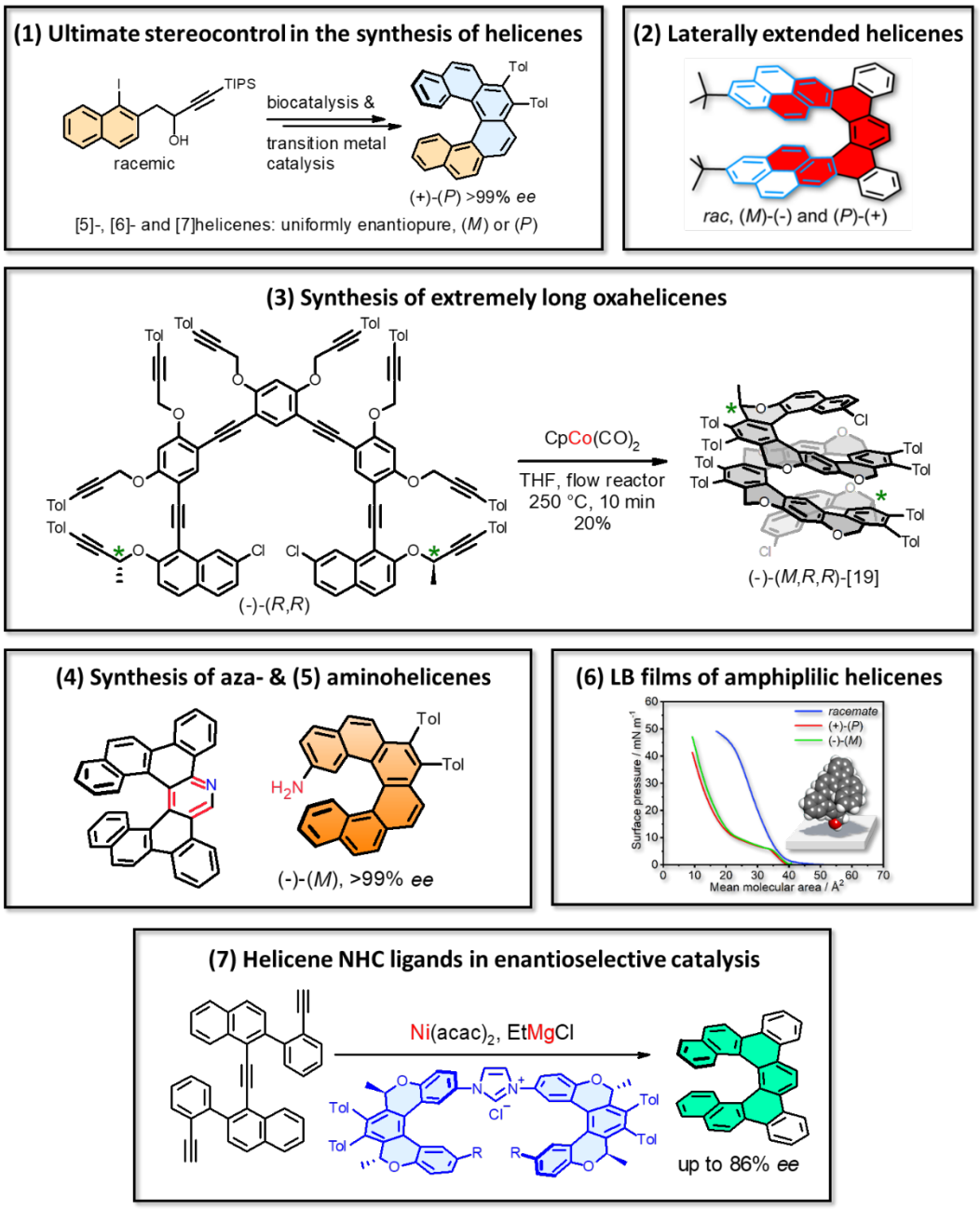
tetrahydrofuran lignans was also developed. The total synthesis efforts are supported by synthetic methodology development to enable efficient and short approaches to new target molecules. A *de novo* approach to new *P*-chiral phosphine ligand lasses was developed. Initial applications in gold-catalyzed cyclization reactions demonstrated that they are highly efficient allowing catalytic reactions at 100 ppm gold complex level (4). These ligands are currently applied in asymmetric catalysis. A versatile photoamination approach for ethers was found, which enables the direct introduction of nitrogen functionality into unfunctionalized ethers. These results form the basis for new photoredox-catalyzed C-H amination reactions, which are even more versatile with respect to substrate scope and applicability. A new area in methodology development consists of finding ways to synthesize non-natural polyfunctional amino acid motifs and explore their ability to be incorporated to peptides and new foldamer architectures, thus enabling addressing these orthogonal functionalities for several purposes.



Selected key publications: (1) Amatov, T.; Pohl, R.; Cisarova, I.; Jahn, U. *Angew. Chem. Int. Ed.* **2015**, *54*, 12153–12157. (2) Dokli, I.; Pohl, R.; Klepetarova, B.; Jahn, U. *Chem. Commun.* **2019**, *55*, 3931–3934. (3) Kapras, V.; Vyklicky, V.; Budesinsky, M.; Cisarova, I.; Vyklicky, L.; Chodounska, H.; Jahn, U. *Org. Lett.* **2018**, *20*, 946–949. (4) Mahamulkar, S. G.; Cisarova, I.; Jahn, U. *Adv. Synth. Catal.* **2018**, *360*, 4215–4224. (5) Hernandez-Guerra, D.; Hlavackova, A.; Pramthaisong, C.; Vespoli, I.; Pohl, R.; Slanina, T.; Jahn, U. *Angew. Chem. Int. Ed.* **2019**, *58*, 12440–12445.

1.3 The Starý group sought to strengthen its expertise in the design, synthesis, and application of non-trivial and non-planar aromatic/π-electron systems. Various aspects of chirality were central to these efforts. The main research topic was the diverse chemistry of helicenes (helically chiral aromatics) with an emphasis on the development of efficient methodologies for their synthesis. Special attention was paid to helicenes that are laterally extended or axially elongated, enantiomerically pure or properly functionalized. Cycloisomerization of alkynes (or nitriles) was central to all these efforts to form the respective (hetero)helicene backbones. Selected helicenes were applied as ligands for metals in enantioselective catalysis.
Helicenes have been extremely difficult targets for stereoselective synthesis for more than half a century. The Starý group discovered how to prepare a wide range of optically pure helicenes from simple starting materials combining bio- and transition metal catalysis (1). A general and ultimate stereocontrol in asymmetric synthesis of fully aromatic helicenes was achieved by employing 1,3-allylic-type strain. Laterally extended helicenes are good examples of an

emerging class of chiral nanographenes. Using Ni^0- or Co^I-mediated [2+2+2] cycloisomerization of dipyrenyl-derived triynes, the Starý group succeeded in incorporating pyrene units into [7]helicene or oxa[7]helicene scaffolds (2). The nonracemic chimerical pyrene-based helicenes can also be obtained through diastereoselective or enantioselective synthesis. A synthetic route to the longest helical oxahelicenes comprising up to 19 fused rings was developed (3). Multiple Co-mediated oligoyne cycloisomerization in a flow reactor was beneficial for folding the precursors into helices. The stereogenic centres in enantiopure oligoynes steered their multicyclization to proceed diastereoselectively. Single-molecule conductivity was studied using the break-junction method. The Starý group paid attention also to the synthesis of *N*-heterocyclic analogues of helicenes and *N*-substituted helicenes. The [2+2+2] cycloisomerization of aromatic cyanodiynes was used in the synthesis of pyridohelicenes and their analogues either in a racemic or enantiopure form (4) and diastereoselective [2+2+2] cycloisomerization of aromatic triynes was used in asymmetric synthesis of nonracemic amino[6]helicenes (5). As far as applications of helicenes are concerned, the chirality-controlled self-assembly of amphiphilic dibenzo[6]helicenes into Langmuir-Blodgett thin films was observed (6). Significant differences were found in the behavior of the Langmuir layers as well as in the morphology (studied by AFM) and (chir)optical spectra of the Langmuir-Blodgett thin films depending on the molecular chirality and nature of the polar group (supported also by molecular dynamics simulations). The precursors of the helical *N*-heterocyclic carbenes were prepared and enantiopure helicene-derived NHC ligands were explored for the first time in enantioselective catalysis (enantioselective cycloisomerization of alkynes) (7). The iconic helicenes and their congeners are currently in the spotlight as a steadily growing number of research groups around the globe recognized their potential for anticipated applications in science and technology.



Selected key publications: (1) Šámal, M.; Chercheja, S.; Rybáček, J.; Vacek Chocholoušová, J.; Vacek, J.; Bednárová, L.; Šaman, D.; Stará, I. G.; Starý, I. *J. Am. Chem. Soc.* **2015**, *137*, 8469–8474. (2) Buchta, M.; Rybáček, J.; Jančařík, A.; Kudale, A. A.; Buděšínský, M.; Vacek Chocholoušová, J.; Vacek, J.; Bednárová, L.; Císařová, I.; Bodwell, G. J.; Starý, I.; Stará, I. G. *Chem. Eur. J.* **2015**, *21*, 8910–8917. (3) Nejedlý, J.; Šámal, M.; Rybáček, J.; Tobrmanová, M.; Szydlo, F.; Coudret, C.; Neumeier, M.; Vacek, J.; Vacek Chocholoušová, J.; Buděšínský, M.; Šaman, D.; Bednárová, L.; Sieger, L.; Stará, I. G.; Starý, I. *Angew. Chem. Int. Ed.* **2017**, *56*, 5839–5843. (4) Klívar, J.; Jančařík, A.; Šaman, D.; Pohl, R.; Fiedler, P.; Bednárová, L.; Starý, I.; Stará, I. G. *Chem. Eur. J.* **2016**, *22*, 14401–14405. (5) Karras, M.; Holec, J.; Bednáová, L.; Pohl, R.; Schmidt, B.; Stará, I. G.; Starý, I. *J. Org. Chem.* **2018**, *83*, 5523–5538. (6) Holec, J.; Rybáček, J.; Vacek, J.; Karras, M.; Bednárová, L.; Budeěšínský, M.; Slušná, M.; Holý, P.; Schmidt, B.; Stará, I. G.; Starý, I. *Chem. Eur. J.* **2019**, *25*, 11494–11502. (7) Sánchez, I. G.; Šámal, M.; Nejedlý, J.; Karras, M.; Klívar, J.;

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2. Nano Chemistry & Nano Science

2.1. The Cígler group has been oriented its research program towards new types of nanoparticles, their nanobiointerface and related materials science. The nanomaterials involved mostly fluorescent nanodiamonds, noble metal-based plasmonic systems and soft matter nanoparticles decorated with synthesized organic or hybrid organic/inorganic architectures. The group reached vast experience in preparation, surface modification and biocompatibilization of fluorescent nanodiamonds. The creation of fluorescent nitrogen-vacancy (NV) centers in diamond lattice was systematically investigated, which resulted in discovery of an original procedure for creation of lattice defects in inorganic nanocrystals using isotropic irradiation with ions generated in situ by a nuclear reaction. This extremely rapid method provides ~1000x higher yields compared to traditional synthetic approaches and creates nanoparticles with narrow emission intensity distribution (1). Combined with innovative synthetic approaches for diamond surface modification such as bench-top radical decarboxylative fluorination (2), the group created targeted bright fluorescent nanodiamond probes operating with high selectivity in biological environment (3). First nanoscale optical sensors monitoring selectively chemical processes on an extremely small scale using quantum sensing approach were designed and synthesized (4). This relaxometric quantum approach was based on measurement of magnetic cross-talk between NV centers in nanodiamonds and Gd(III)-complexes present in polymer interface engineered on the nanoparticles. Monitoring of localized chemical processes occurring on an extremely small scale (down to ~10^{-22} mol; corresponds to tens of molecules) was achieved. Finally, a new nanodiamond interface based on supported lipid bilayers was developed which led to a further increase of detection sensitivity about one order of magnitude (5). The Cígler group was responsible for conceiving projects, designing experiments, performing all synthetic and preparative tasks, and contributed to interpreting experimental results (spectroscopic, quantum sensing, and biological data).

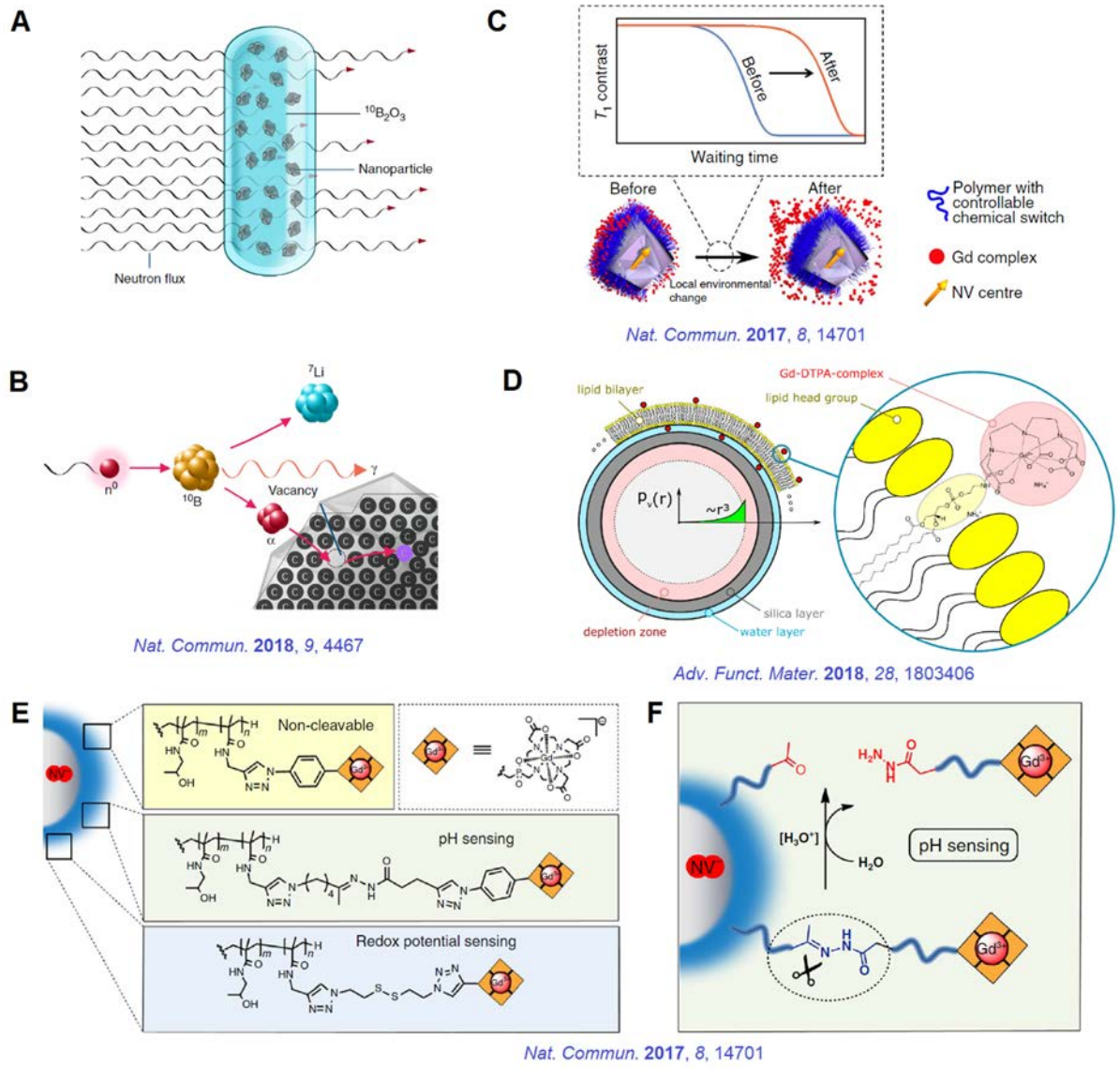
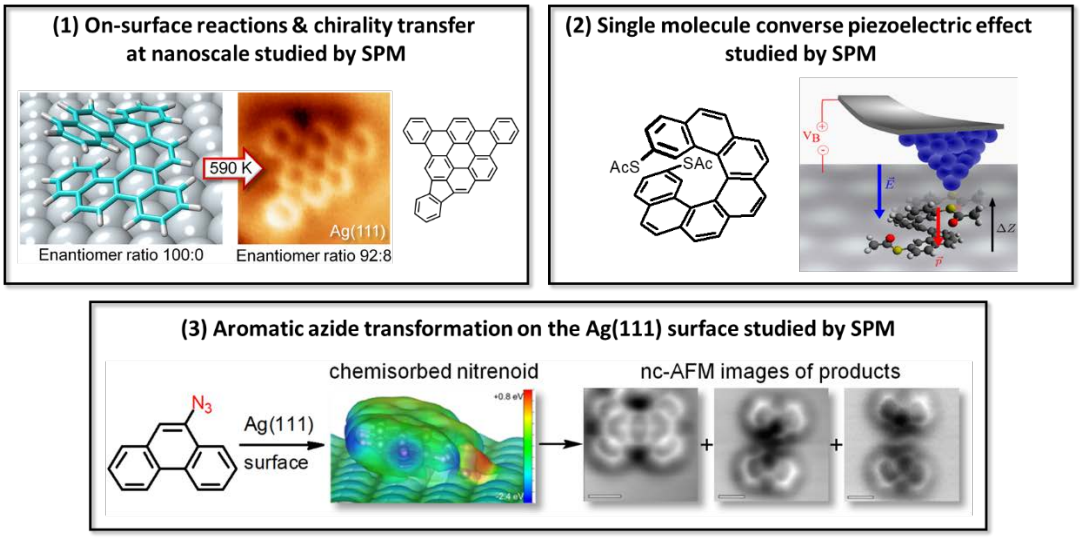


Figure: *(A) Basic principle of the creation of lattice defects in inorganic nanocrystals with ions generated in situ by a nuclear reaction. The nanoparticles are embedded in a glassy melt of $^{10}B_2O_3$ exposed to a neutron flux. (B) Detail of α particles and $^7Li^+$ ions formed in situ by ^{10}B neutron capture entering a nanodiamond particle and creating vacancies inside. (C) The sensing mechanism of a hybrid quantum nanosensor consisting of nanodiamond and a polymer interface. The Gd-complexes attached to the polymer are selectively cleavable in response to a local change of environment. The release of the complexes is optically monitored using T_1 relaxometry. (D) A nanodiamond particle coated with silica shell is further encapsulated in a biomimetic supported lipid bilayer. A Gd-modified lipid present in the bilayer acts as a spin probe communicating magnetically with NV centers in nanodiamond. (E) Chemical structure of the polymer interface of a nanodiamond quantum sensors equipped with Gd-complexes attached via a non-cleavable and two types of cleavable linkers. (F) Example of the specific release mechanism for pH-dependent, hydrolytically cleavable linker.*

Selected key publications: (1) Havlik, J.; Petrakova, V.; Kucka, J.; Raabova, H.; Panek, D.; Stepan, V.; Zlamalova Cilova, Z.; Reineck, P.; Stursa, J.; Kucera, J.; Hruby, M.; Cigler, P. *Nat. Commun.* **2018**, *9*, 4467. (2) Havlik, J.; Raabova, H.; Gulka, M.; Petrakova, V.; Krecmarova, M.; Masek, V.; Lousa, P.; Stursa, J.; Boyen, H.-G.; Nesladek, M.; Cigler, P. *Adv. Funct Mater.* **2016**, *26*, 4134–4142. (3) Slegerova, J.; Hajek, M.; Rehor, I.; Sedlak, F.; Stursa, J.; Hruby, M.;

Cigler, P. *Nanoscale* **2015**, *7*, 415–420. (4) Rendler, T.; Neburkova, J.; Zemek, O.; Kotek, J.; Zappe, A.; Chu, Z.; Cigler, P.; Wrachtrup, J. *Nat. Commum.* **2017**, *8*, 14701. (5) Vavra, J.; Rehor, I.; Rendler, T.; Jani, M.; Bednar, J.; Baksh, M. M.; Zappe, A.; Wrachtrup, J.; Cigler, P.: *Adv. Funct. Mater.* **2018**, *28*, 1803406.

2.2 The Starý group closely cooperated with the group of Pavel Jelínek at the Institute of Physics AS CR to investigate basic chemical and physical processes at the single molecule level using a combination of advanced molecular design, organic synthesis and state-of-the-art UHV scanning probe microscopy techniques. The joint team observed a chemical transformation of individual heptahelicene molecules on the Ag(111) surface and demonstrated chirality transfer during the multistep reaction (1). They took advantage of the latest advances in scanning probe microscopy, which made it possible to determine the chemical bond between individual atoms and thus assign the molecular structure and chirality. Furthermore, they demonstrated for the first time the converse piezoelectric effect on a single molecule of sulfanylated heptahelicene on the Ag(111) surface (2). The piezoelectric constant calculated from the experimental data was significantly larger than at piezoelectric polymers and the origin of the effect was explained by quantum mechanical calculations. In the quest for identifying useful on-surface reactions triggered by heat (or light), they turned attention to aromatic azides whose transformation on the Ag(111) surface was studied by scanning probe microscopy techniques (3). High-resolution imaging supported by first-principle calculations revealed the structure of the prevalent products that pointed to their origin in a common and elusive aromatic nitrenoid intermediate chemisorbed on the Ag(111) surface. The Starý group was responsible for conceiving projects, designing experiments, synthesizing molecular models, performing selected theoretical calculations and interpreting experimental results (chemical transformations).



Selected key publications: (1) Stetsovych, O.; Švec, M.; Vacek, J.; Vacek Chocholoušová, J.; Jančařík, A.; Rybáček, J.; Kosmider, K.; Stará, I. G.; Jelínek, P.; Starý, I. *Nat. Chem.* **2017**, *9*, 213–218. (2) Stetsovych, O.; Mutombo, P.; Švec, M.; Šámal, M.; Nejedlý, J.; Císařová, I.; Vázquez, H.; Moro-Lagares, M.; Berger, J.; Vacek, J.; Stará, I. G.; Starý, I.; Jelínek, P. *J. Am. Chem. Soc.* **2018**, *140*, 940–946. (3) Hellerstedt, J.; Cahlík, A.; Stetsovych, O.; Švec, M.; Shimizu, T. K.; Mutombo, P.; Klívar, J.; Stará, I. G.; Jelínek, P.; Starý, I. *Angew. Chem. Int. Ed.* **2019**, *58*, 2266–2271.

3. Materials Chemistry

3.1. The Michl group addressed three themes related to materials chemistry and a fourth fundamental organic/inorganic theme in the chemistry of boron and fluorine. In the order of importance, the themes were (i) the development of singlet fission into a practical tool for increasing the efficiency of solar cells, where we cooperated with the groups of Havlas at IOCB, Ludvík at the Heyrovský Institute, Pfleger at the IMC, MacQueen at the Humboldt-Zentrum and Kaupp at the Technical University, both in Berlin, Germany, Broer at the University of Groningen in the Netherlands, and Kahr at New York University, Johnson at NREL in Golden, Colorado, and Michl's group at the University of Colorado Boulder, all three in USA; (ii) the production and characterization of sulfur-free alkyl monolayers on gold that promise many applications in nanoscience, in cooperation with Bastl at the Heyrovský Institute; and (iii) the effort to fabricate ferroelectric surfaces for nanoelectronics from dipolar molecular rotors jointly with Batail at the University of Angers, France, Feringa at the University of Groningen in the Netherlands, and Rogers at the University of Colorado Boulder, USA. The effort in boron-fluorine chemistry (iv) focused on carboranes was joint with Michl's group in Colorado and with Miller at Brookhaven National Laboratory, New York, USA.

Singlet Fission (SF; GAČR 19-22806S): Practical use of SF, which promises to raise the theoretical maximum efficiency of solar cells from 1/3 to close to 1/2, requires a currently hypothetical organic material containing chromophores with first triplet (singlet) excitation energies of ~1.1 (~2.2) eV packed appropriately in the solid, carrying an interface with another material capable of converting triplet excitons into pairs of charges collectable at electrodes, and stable under irradiation. At the outset, it was not known what "packed appropriately" actually means and our main accomplishment in this period was to figure it out from first principles and publish a public domain program for the prediction of molecular geometries that maximize the rate of SF(1). We have also worked on the development and testing of new chromophores for SF. On the one hand, we try to find chromophores that are very lightfast and could be used in practice, and on the other hand, we are looking for those whose molecules are small enough for high-level theoretical treatment. We have published many but not yet all these results. An example from the first category is the very stable commercial dye cibalackrot, which we examined in great detail (2) and deduced the needed changes in the crystal structure as a guide for crystal engineering. Examples from the second category are derivatives of 2,3-diamino-1,4-naphthoquinone and the cyclopeptide biradicaloid shown on the right in **Figure 1**. An attempt to prepare the parent with $R_1 = R_2 = H$ showed that it is unstable to dimerization and oligomerization and that larger substituents R will be required. A third direction in our SF effort addresses the splitting of excitons into charges. We are developing a prototype hybrid SF solar cell in which silicon surface is functionalized with a monolayer of SF chromophore **1** [initially, a functionalized 9,10-bis(phenylethynyl)anthracene] attached to the surface covalently through a conjugating double or triple bond and coated with a layer of SF chromophore **2** [initially, 9,10-bis(phenylethynyl)anthracene]. The function of the layer of **1** is to facilitate the transfer of excitons generated by SF in the layer of **2** into the underlying layer of silicon. The remaining silicon atoms at the surface will be passivated with inert groups such as methyl (**Figure 2**). The synthetic work has been completed and we are currently working on the covalent attachment of **1** to the silicon surface.

Gold Surface Alkylation: We have followed up our initial discovery that contact with alkylstannane solutions produces alkyl monolayers on a gold surface with an investigation of their structure and properties and the reaction scope and mechanism. We found that the monolayers contain not only alkyl groups but also tin oxides and are mechanically but not electrically insulating. The main publication so far (3) summarizes these results and two more are under preparation. They contain a spectroscopic proof of the presence of Au-C bonds using the ^{13}C shift in the surface Raman spectrum of an attached butyl group and propose a detailed mechanism for the surface alkylation reaction based on observations and on density functional theory calculations. An important part of the evidence is the identification of the byproducts of the self-limiting surface reaction, which is rarely if ever performed, and was unusually demanding because of the minute amounts involved. An as yet incomplete study

describes the use of alkylstannanes modified on the tin atom, which alkylate gold surfaces without depositing tin oxides.

Molecular Rotors: We continued our exploration of the properties of assemblies of dipolar molecular rotors carried by surfaces or thin layers and found out much about their behavior, including evidence of ferroelectric interactions, but observed no actual ferroelectric phase. The so far unprecedented achievement of a true ferroelectric surface phase by self-assembly may well be impossible, primarily because of excessive structural defects, inherent to this approach. The main publication from this endeavor (4) intimates a change in direction that acknowledges this reality and redirects this research toward the fabrication of light-driven self-propelling nanoparticles.

Boron and Fluorine Chemistry: Here, our efforts have been centred at the exploratory chemistry of *closo*-carborane anions derived by substitution from $CB_{11}H_{12}(-)$ and the related radicals, $CB_{11}H_{12}(\bullet)$ (5). Several of these redox couples were found to be reversible and many of their oxidation potentials are extraordinarily high, one more than 4V above Fc/Fc^+. This blue radical $HCB_{11}F_5(CF_3)_6(\bullet)$ is the strongest neutral oxidant known. Other results include a remarkable collision-induced fragmentation of charged carborenes, analogues of the organic benzyne, electronic conjugation between neutral carborane cages $C_2B_{10}H_{12}$ and their unusual electrochemistry, synthesis of borenium ylides and other uncommon structures, and the use of $CB_{11}H_{12}(-)$ for the solubilization of Li^+ in non-polar solvents, permitting it to catalyze alkene polymerization to very highly branched polymers.

Figure 1. Possible routes to biradicaloids.

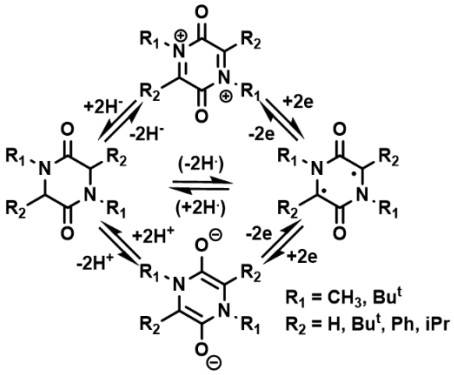
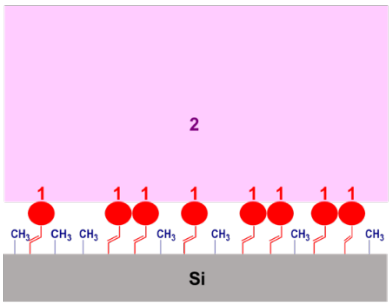


Figure 2. Design of hybrid solar panel.



1: A monolayer of SF chromophore **1**
2: A layer of SF chromophore **2**

Selected key publications: (1) Zaykov, A.; Felkel, P.; Buchanan, E. A.; Jovanovic, M.; Havenith, R. W. A.; Kathir, R. K.; Broer, R.; Havlas, Z.; Michl, J. *J. Am. Chem. Soc.* **2019,** *141*, 17729–17743. (2) Ryerson, J. L.; Zaykov, A.; Suarez, L. E. A.; Havenith, R. W. A.; Stepp, B. R.; Dron, P. I.; Kaleta, J.; Akdag, A.; Teat, S. J.; Magnera, T. F.; Miller, J. R.; Havlas, Z.; Broer, R.; Faraji, S.; Michl, J.; Johnson, J. C. *J. Chem. Phys.* **2019,** *151*, 184903. (3) Kaletová, E.; Kohutová, A.; Hajduch, J.; Kaleta, J.; Bastl, Z.; Pospíšil, L.; Stibor, I.; Magnera, T. F.; Michl, J. *J. Am. Chem. Soc.* **2015,** *137*, 12086–12099. (4) Kaleta, J.; Chen, J.; Bastien, G.; Dračínský, M.; Mašát, M.; Rogers, C. T.; Feringa, B. L.; Michl, J. *J. Am. Chem. Soc.* **2017,** *139*, 10486–10498. (5) Wahab, A.; Douvris, C.; Klíma, J.; Šembera, F.; Ugolotti, J.; Kaleta, J.; Ludvík, J.; Michl, J. *Inorg. Chem.* **2017**, *56*, 269–276.

Research activity and characterisation of the main scientific results

The Konvalinka group studies well-established therapeutic and diagnostic markers as well as explores novel pathways to combat viral replication and tumor growth. The former targets involve the s HIV protease and the complex process of HIV processing and maturation, or glutamate carboxypeptidase II, a cancer marker and a neuropeptidase. The latter involves protein-protein interaction of the subunits of influenza polymerase or novel, poorly characterized proteins with potential proteolytic activity, such as DNA damage-inducible protein 1 and 2. We were able to provide structural and functional characterisation of these protein targets, develop assays for the analysis of their activity, design specific inhibitors and followed the molecular evolution of theses proteinsunder the selection pressure of specific drugs.

In order to visualize and quantify our target proteins, the group recently developed synthetic antibody-like polymer scaffolds containing a specific ligand of the particular protein ("molecular address"), affinity anchor (typically biotin moiety), and an imaging marker (fluorescent probe) attached to a hydrophilic copolymer. This versatile, easy to assemble scaffold called iBody is able to replace a monoclonal antibody in a number of in vitro and in vivo applications. Furthermore, they developed a novel assay for detecting enzymes as diagnostic markers and identifying enzyme inhibitors in drug development. The system called DIANA enables quantification of zeptomolar amounts of enzymes and high-throughput screening of potential inhibitors. Both the method open number of potential applications that are currently being studied.

Selected key publications:

Šimon, P. et al. ***ACS Chem. Biol*.** (2018), 13: 3333–3342; Kožíšek, M. et al. ***Biochem. J.*** (2018), 475: 3847–3860; Dvořáková, P. et al. ***J. Med. Chem*.** (2017), 60: 8385–8393; Navrátil, V. et al. ***Nucleic Acids Res*.** (2017), 45, e10; Šácha, P. et al. ***Angew. Chem., Int. Ed.*** (2016), 55: 2356–2360; Schimer, J. et al. ***Nat. Commun.*** (2015), 6, 6461.

The Pichová group studied retroviral assembly and maturation using model retroviruses M-PMV, MMTV and also HIV and identified structural motifs that are important for assembly of immature particles and their re-arrangement into mature viral particles. By combination of crystallization, molecular dynamics simulations, and experiments with mutated MMTV matrix indicated the unique role of myristylation in matrix oligomerization and assembly. The group also developed a rapid and simple high-throuoghput method for HIV assembly monitoring. This method is suitable for testing of libraries of potential HIV assembly inhibitors. In mycobacteria research, the group worked on contribution of enzymes from central carbon metabolism and nucleotide biosynthesis to metabolic switch between replicative and non-replicative state, which is typical for latent infection. Particularly, phosphoenolpyruvate kinase and pyruvate kinase were structurally and kinetically characterized and results indicated that hypoxia-triggered non-replicating conditions in bacteria, such as increased reducing environment, decreased pH, decreased ATP level, and different composition of metabolites significantly influence enzyme activities and potentially contribute to changes in metabolic fluxes. Using the insect models, the group found that a single amino acid in the membrane fatty acid desaturase significantly changes specificity of this enzyme, which participates in introduction of double bonds in fatty acids. Further a novel fatty acyl reductase family, whose members dispaly unusual broad specificity was discovered. These results contribut both, to evolutionary study of pferomones as well as to application in biotechnology.

Selected key publications:

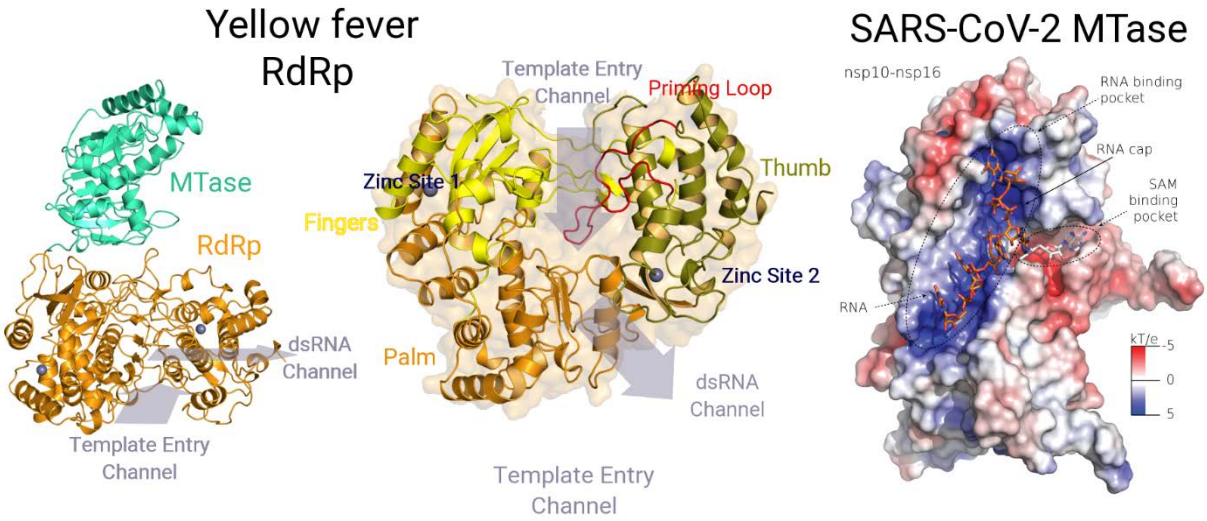
Schur, F.K.M. et al. ***Nature*** (2015), 517: 505-508; Doležal, M. et al. ***J. Biol. Chem***. (2016), 291 (39): 20630-20642; Doležal, M. et al. ***Retrovirology*** (2016), 13; Hadravová, R. et al. ***Virology*** (2015), 486, 78-87; Machová, I. et al. ***PLoS One*** (2017), 12 (1); Snášel, J. et al. (2019) BBA 2, 124-139; Buček, A. et al. ***Proc. Natl. Acad. Sci. U.S.A.*** (2015), 112, 12586-591; Tupec, M. et al. ***eLife*** (2019), 8 (4).

The Mareš group conducted research on pathology-associated cathepsin proteases and their inhibitors, with main topics focused on blood-feeding parasites and selected human diseases. Parasitic blood flukes *Schistosoma* are the cause of schistosomiasis, which afflicts 240 million people worldwide. Based on mapping of the proteolytic system of schistosomes, several proteases were identified as promising targets for chemotherapy (in collaboration with University of California San Francisco and San Diego). Two of them, SmPOP and SmSP2, were found to play an important vasoregulatory and anti-hemostatic roles in parasite-host interactions, and the first inhibitors of SmPOP effective against live schistosomes were developed. *Ixodes* ticks are vectors of Lyme disease and encephalitis, and their proteolytic system is a vaccination target and source of pharmacologically active proteins. 3D structures and functions were determined for Iristatin, an immunosuppressive protease inhibitor, and the cathepsin IrCD1, which is regulated by the first endogenous inhibitors of aspartic proteases discovered in higher organisms (in collaboration with the Institute of Parasitology, CAS). Finally, several projects were initiated to identify natural structural motifs for development of biomimetic inhibitors against medically relevant cathepsins and related proteases. Namely, cathepsin inhibitors from plant Kunitz family were analyzed, and specific inhibitors were designed for cathepsins associated with cancer and osteoporosis.

Selected key publications:

Fajtová, P. et al. ***PLoS Negl. Trop. Dis.*** (2015), 9 e0003827; Leontovyč, A. et al. ***PLoS Negl Trop. Dis.*** (2018), 12, 1-26; Hánová, I. et al. ***Cell Chem. Biol.*** (2018), 25, 318-329; Kotál, J. et al. ***Cell Mol. Life Sci.*** (2019), 76, 2003-2013; Srp, J. et al. ***Insect Biochem. Mol. Biol.*** (2016), 78, 1-11; Li, M. et al. ***Acta Crystallogr. D Struct. Biol.*** (2019), 75, 56-69; Guo, J. et al. ***J Struct Biol.*** (2015), 192, 554–560.

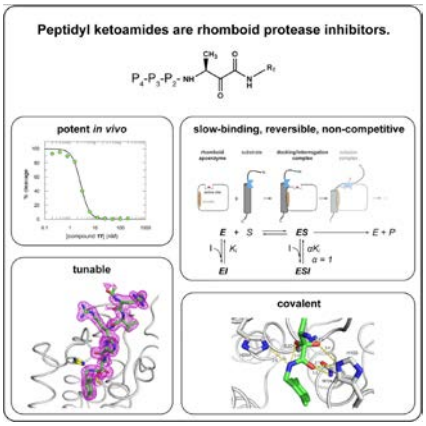
The Bouřa group structurally characterized enzymes from several important human +RNA viruses. The Boura group published the first paper with an enzymatic characterization of the Zika polymerase and also crystallized the MTase from the Zika flavivirus in a complex with the pan-MTase inhibitor sinefungin. The group has also managed to solve one of the very few crystal structure of a full length flaviviral polymerase, the Yellow fever RdRp that revealed a conserved hotspot for drug design shared among flaviviruses. Very recently, the Boura group also structurally and functionally characterized several protein complexes from the new SARS-CoV-2 virus. The crystal structure of the primase nsp7:nsp8 complex was solved at 2.0 Å resolution and revealed a putative RNA binding site. Most importantly, the group also solved the structure of the SARS-CoV-2 2'-*O* MTase complex nsp10:nsp16 explaining how this coronavirus masks its RNA from innate immunity.

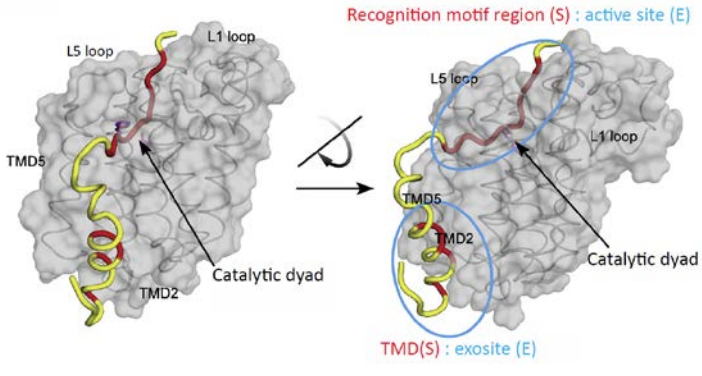


Selected key publications:

Hercík, K. et al. ***Antivir. Res.*** (2017), 137, 131-133; Hercík, K. et al. ***Arch. Virol.*** (2017), 162, 2091-2096; Dubánková A. et al. ***Antivir. Res.*** (2019), 169, 104536; Konkolová, E. et al. ***J. Struct. Biol.*** (2020), 107548; Krafčíková, P. et al. ***Nat. Commun.*** (2020), 11, 3717.

The Stříšovský group has continued their studies of intramembrane proteolysis and its cell biological roles. Since 2015, the group has developed the first highly potent, selective and pharmacologically compliant inhibitors of rhomboid proteases, which were then used to discover a new membrane protein quality control mechanism in bacteria that is evolutionarily related to eukaryotic ER-associated degradation (ERAD). We continued our work on the structure and mechanism of rhomboid superfamily proteins that are key for the retrotranslocation of proteins across membranes during ERAD and related processes. Finally, we have recently ventured into a new exciting area of membrane protein biogenesis and folding by starting to work on the ER membrane protein complex (EMC) within an international consortium (PT, UK, CZ). The Strisovsky group successfully passed their international evaluation at IOCB in 2019, and as a result was promoted to a Senior Research Group (tenured).





Selected key publications:

Tichá, A. et al. ***J. Biol. Chem***. (2017), 292, 2703-2713; Tichá, A. et al. ***Cell Chem. Biol.*** (2017), 24, 1523-1536; Stříšovský, K. et al. ***United Kingdom Patent application No. GB1709317.0*** (2017); Began, J. et al. ***EMBO J.*** (2020), 39, e102935; Liu, G. et al. ***EMBO J.*** (2020), 39, e102922;

Oikonomidi, I. et al. ***eLife*** (2018), 7, e35032; Tichá, A. et al. ***Trends Biochem. Sci.*** (2018), 43, 726-739.

The Mertlíková group has participated in several drug discovery projects focused on inflammatory bowel disease, leukemias and steroidal targets (NHRs, NMDA receptors). Some of the data remain undisclosed for IP protection reasons, many other have been presented elsewhere in this report by our collaborators from IOCB Chem clusters (Medicinal Chemistry). To exemplify the diverse research activities of the group, we have been able to identify mechanism by which 9-norbornylpurine derivatives induce apoptosis in tumor cells i.e. *via* depletion of cellular glutathione thus inducing oxidative stress and endoplasmic reticulum stress response.

Selected key publications:

Plačková, P. et al. ***Free Radic. Biol. Med.*** (2016), 97, 223-235.

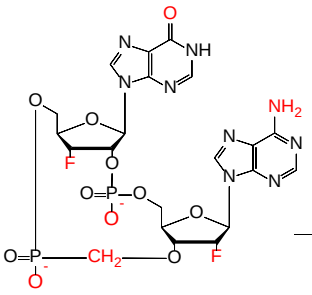
The Weber group identified protein arginine methyltransferase 5 (PRMT5) as a potent controller of Hepatitis B virus (HBV) core protein cell trafficking and function. They demonstrated that PRMT5 directly interacts with the HBV core (HBc) protein and dimethylates arginines at the carboxyl-terminus. Mass spectrometry analysis of HBc showed several potential methylation, phosphorylation and ubiquitination sites. The HBc symmetric dimethylation was linked to serine phosphorylation and resulted in increased accumulation of HBc in nuclei. On the contrary, the monomethylated HBc retained in the cytoplasm.
In the area of nanoparticle research, Weber group was part of multinational collaboration that developed broad-spectrum virucidal nanoparticles. We showed that gold nanoparticles coated with undecanesulfonic acid-containing ligands (Au-MUS) mimicking heparan sulfate proteoglycan receptor potently inactivate dengue virus. These modified nanoparticles bind to the virus in multivalent fashion that leads to local distortions and eventually to a global virus deformation resulting in irreversible loss of infectivity.

Selected key publications:

Lubyová, B. et al. ***PLoS One*** (2017), 12, e0186982; Cagno, V. et al. ***Nat. Mater.*** (2018), 17, 195-203.

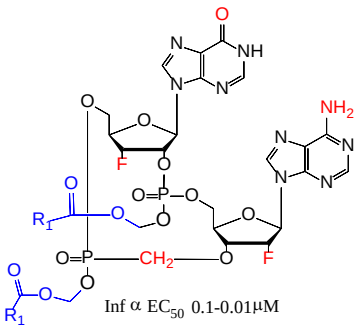
The Birkus targeted research group is currently focussed on identification of novel agonists and inhibitors of cGAS-STING pathway which plays an important role in antiviral and antitumor immune responses, but can also trigger various auto-inflammatory diseases. In regard of agonists we identified seven novel series of cyclic dinucleotides (CDNs) with improved properties compared to the natural STING agonists. First, we replaced phosphodiester bonds in the natural CDNs with carbon-phosphate bonds that are resistant toward hydrolysis by host phosphodiesterases. We prepared CDNs by conventional chemical synthesis, but we also used an enzymatic synthesis by employing vertebrate and bacterial dinucleotide cyclases. Second, we masked negative charges on CDN phosphates with bio-labile lipophilic prodrug moieties that allow free diffusion of CDNs across cellular membranes. As a consequence of this effort we were able to prepare compounds that were two – three orders of magnitude more potent *in vitro* than natural CDNs. Furthermore, the compounds showed potent anti-HBV and anti-tumoral activity *in vivo* and induced adaptive immune responses toward viral and cancer antigens *in vivo*. This project was originally performed in collaboration with a large pharma company Gilead Sciences (USA) and resulted in thirteen international patent applications.

Another project deals with identification of cGAS inhibitors and is done in collaboration with Centre for Drug Design and Discovery (Leuven Belgium). Here we performed virtual and biochemical screening of large libraries of compounds and identified two classes of inhibitors which activity was further improved 100 fold through medicinal chemistry effort. Currently we continue lead optimizations with a goal to have a preclinical candidate by the end of 2021. At this stage we cannot reveal the structures due to pending patent applications.



Inf α EC_{50} 100-200μM

WO 2019193533 A1
WO 2019193542 A1
WO 2019193543 A1



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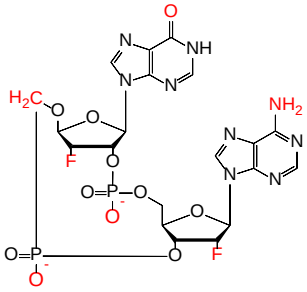
WO 2020178769 A1
WO 2020178770 A1



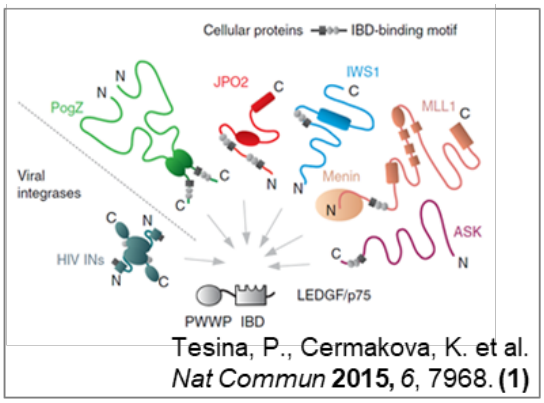
US 20190185510 A1
US 20190185509 A1
US 20190183917 A1

Selected key publications:

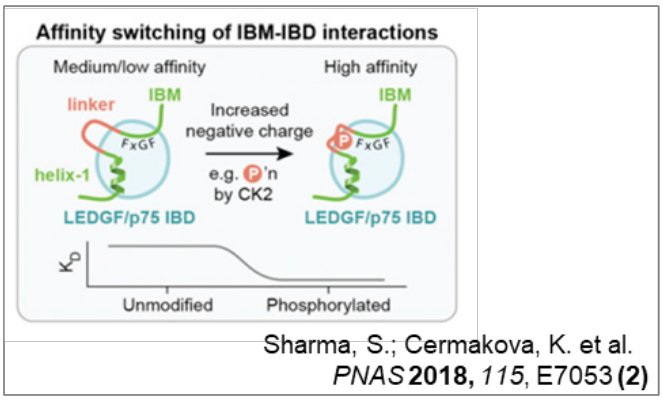
Novotná, B. et al. ***J. Med. Chem.*** (2019), 62, 10676-10690.

Research activity and characterisation of the main scientific results

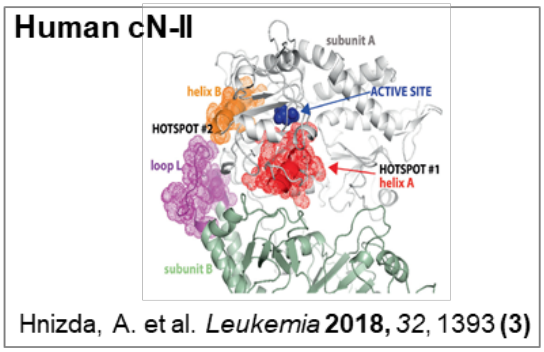
The **Maloy Řezáčová group** studied the interaction network of the epigenetic reader LEDGF/p75 that is alongside its physiological role implicated also in HIV infection and development of a particular subtype of an aggressive childhood leukaemia. Using structural biology, we revealed that the LEDGF/p75 interactome is maintained by an intrinsically disordered binding motif common to all known cellular partners (1) and that this binding is modulated by a controlled phosphorylation of the interaction motif (2). We also contributed to detailed analysis of cancer missense mutations in SMARCA4, the ATPase subunit of BAF chromatin remodelling complexes (3). Our work revealed molecular mechanism of hyperactivation cytosolic 5 -nucleotidase (cN-II) and provided the basis for targeting this enzyme in relapsed acute lymphoblastic leukaemia (4, 5). In (6) we developed an approach for characterization of transient dynamic protein-ligand complexes by combining paramagnetic nuclear magnetic resonance with quantum chemical calculations and molecular dynamics simulations.
During the evaluated period we used structure-assisted design in several projects targeting medicinally relevant human enzymes (7, 8). Most significant project, done in collaboration with Institute of Inorganic Chemistry and Institute of Molecular Genetics CAS and Institute of Molecular and Translational Medicine, Olomouc, developed and characterized series a large series of inhibitors of human carbonic anhydrase based on sulfamides and sulphonamides incorporating carborane clusters. Some compounds demonstrated favourable *in vitro* toxicology and pharmacokinetics profiles and reduced tumour size in mice (9). This series of compounds also became the subject of successful patent applications (EP12786800.8, 16/3/2016; US9,290,529B, 22/3/2016).



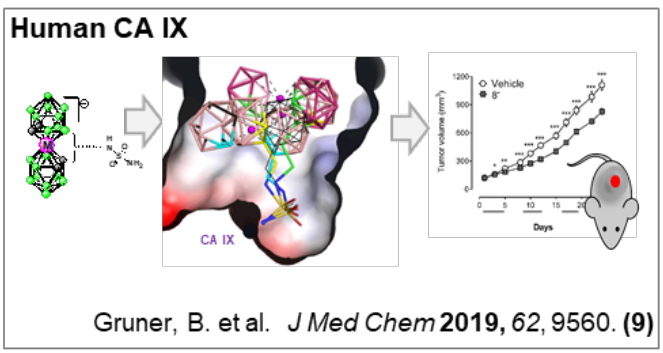
Tesina, P., Cermakova, K. et al. *Nat Commun* **2015,** *6*, 7968. **(1)**



Sharma, S.; Cermakova, K. et al. *PNAS* **2018,** *115*, E7053 **(2)**



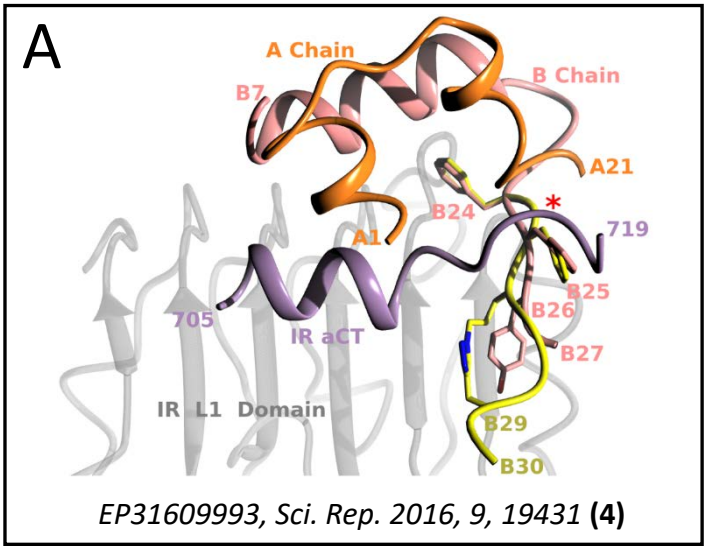
Hnizda, A. et al. *Leukemia* **2018,** *32*, 1393 **(3)**



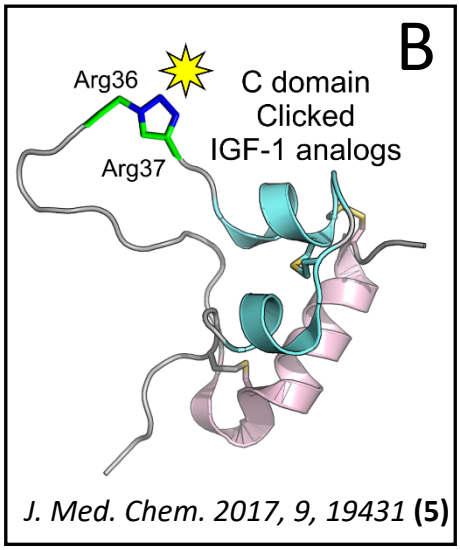
Gruner, B. et al. *J Med Chem* **2019,** *62*, 9560. **(9)**

Selected key publications of the Maloy Řezáčová group: (1) Tesina, P., Cermakova, K. et al. *Nat Commun* **2015,** *6*, 7968. (2) Sharma, S.; Cermakova, K. et al. *Proc Natl Acad Sci U S A* **2018,** *115*, E7053. (3) **Hodges, H. C. et al.,** *Nature Struct Mol. Biol.* ***2018****, 25, 61*. (3) Hnizda, A. et al. *Leukemia* **2018,** *32*, 1393. (5) Hnizda, A. et al. *BMC Biol* **2016,** *14*. (6) Srb, P. et al., *Phys Chem Chem Phys* **2019,** *21*, 5661. (7) Pachl, P. et al. *Eur J Org Chem* **2018,** 5144. (8) Pachl, P. *Medchemcomm* **2015,** *6*, 1635. (9) Gruner, B. et al. *J Med Chem* **2019,** *62*, 9560.

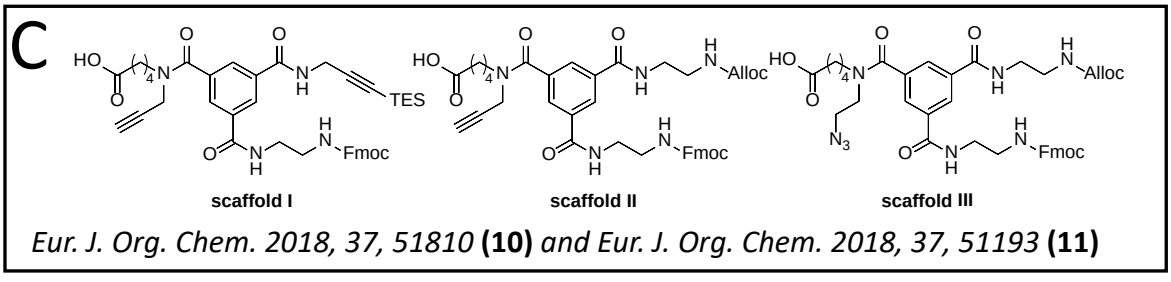
The **Jiráček group** has continued in structure-activity studies aiming to decipher structural motifs in insulin, IGF-1 and IGF-2 that determine their different receptor binding specificities and biological activities. The most important results were as follows: i) Biological testing and NMR structural characterization of recombinant IGF-2 analogues specifically modified in the C-domain that is absent in insulin enabled to identify amino acids that are behind differential affinity of IGF-2 for insulin and IGF-1 receptors (1). ii) Biological activities of insulin-IGF hybrid molecules revealed that addition of amino acids to the insulin's B-chain C-terminus can change hormones' binding affinity in favour of metabolic IR-B receptor (2). iii) Synthesis and characterization of a large series of insulin analogues specifically intra-chain cross-linked with a triazole bridge yielded insulin analogue with a significant preference for metabolic IR-B isoform of the insulin receptor (3) (Fig. A). The most active and IR-B selective cross-linked insulin analogues were patented (4). iv) A new method for the total chemical synthesis of 70-amino acid long IGF-1 hormone was developed (5). This methodology entails the ligation of precursor chains by the Cu-catalysed azide-alkyne cycloaddition (Fig. B) and represents a convenient synthetic platform for production of nonstandard IGF-1 analogues that are inaccessible by standard peptide synthesis. v) IGF-1/2 analogues with exceptionally high binding affinities for insulin receptors (6). vi) An insulin analogue with unprecedented extremely high binding affinity for the IGF-1 receptor was prepared by mutation at just three insulin positions (7). vii) A large and systematic study involving synthesis of mutants (at four different sites) of three hormones (insulin, IGF-1 and IGF-2) and testing of mutants with three different receptors (IR-A, IR-B and IGF-1R) revealed that equivalent mutations in the hormones are receptor-specific and have differential biological effects (8). viii) In a quest for insulin mimetics, three new tri-orthogonal scaffolds that enable synthesis of three different peptides on the same scaffold were developed (9,10) (Fig. C) and model compounds that can effectively bind insulin receptor were prepared (10). These results open up new avenues for combinatorial libraries of hormone mimetics.



EP31609993, Sci. Rep. 2016, 9, 19431 **(4)**



J. Med. Chem. 2017, 9, 19431 **(5)**



Eur. J. Org. Chem. 2018, 37, 51810 **(10)** *and Eur. J. Org. Chem. 2018, 37, 51193* **(11)**

Selected key publications of the Jiráček group: (1) Hexnerova, R. et al. *J Biol Chem* **2016**, 291, 21234. (2) Krizkova, K. et al. *Biochemistry* **2016**, 55, 2903. (3) Vikova, J. et al. *Sci Rep* **2016**, 6, 19431. (4) European patent EP31609993, **2018**. (5) Machackova, K. et al. *J Med Chem* **2017**, 60, 10105. (6) Machackova, K. et al. *Biochemistry* **2018**, 57, 2373. (7) Chrudinova, M. et al. *J Biol Chem* **2018**, 293, 16818. (8) Machackova, K. et al. *J Biol Chem* **2019**, 294, 17371. (9) Vaněk, V. et al. *Eur J Org Chem* **2015**, 17, 3689. (10) Picha, J. et al. *Eur J Org Chem* **2018**, 37, 5180. (11) Fabre, B. et al. *Eur J Org Chem* **2018**, 37, 5193.

The **Maletínská group studied** regulation of food intake and processes related to obesity, diabetes and neurodegeneration, specifically in the following three projects: (i) Prolactin-releasing peptide (PrPR) analogues in metabolic syndrome. PrPR is a new anorexigenic neuropeptide; both PrRP and its receptor knock-outs are obese. Anorexigenic neuropeptides are potential anti-obesities but their peptide character complicates their delivery to brain, necessary for their exclusively central effect. This project aims to select effective PrRP analogs modified with fatty acid that would prolong their half-life and enable them to cross blood brain barrier (1). PrRP analogues were subjected to binding and functional tests in cells with relevant receptors (2). The most potent agonists, palmitoylated in N-terminal part, were tested in mice and rats for short-term effect on food intake, and for long-term effects in mouse and rat models with diet-induced obesity and related disorders such as type 2 diabetes and hypertension, called metabolic syndrome (3-5). We showed that lipidization of PrRP might be an effective way to deliver peripherally peptides with central effects for a potential treatment of metabolic syndrome. Metabolomics in urine and plasma was also performed in cooperation with Institute of Microbiology (6). (ii) Neuroprotective effects of novel analogues of anorexigenic prolactin-releasing peptide (PrRP) in mouse models of neurodegeneration and obesity. Type 3 diabetes is more known as Alzheimer's disease. Alzheimer's disease is the most common form of dementia and despite of the effort of many research groups the mechanism leading to its development still remains unknown. In our group we study the possible connection between Alzheimer's disease development and type 2 diabetes. Type 2 diabetes can cause the resistance to the effect of insulin in brain where insulin is important for memory formation and learning. Insulin resistance then leads to the development of neuropathological changes which are the hallmarks of Alzheimer's disease (for example increased phosphorylation of Tau protein). These pathological changes are studied in mouse and cellular models. The major aim of this project was to find out if novel anorexigenic peripherally administered and centrally acting analogs of prolactin-releasing peptide (PrRP) have similar beneficial effect on the mentioned neurodegenerative changes in several mouse models of neurodegeneration and their common aged wild type (in cooperation with University of Lancaster and INSERM, Lille) (7-9). Implication of PrRP analogs on spatial memory were also assessed. Lipid changes related to neurodegeneration in brain were studied using mass spectrometry imaging in cooperation with Mass spectrometry department at IOCB (10). Besides, effects of PrRP analogs on induced neuronal damage in cultured neurons were investigated (11). We have proven that our novel PrRP analogs have potent beneficial effects on neurodegenerative changes and memory. (iii) Long-acting ghrelin agonists: impact on metabolic and cardiovascular parameters in experimental cachexia and inflammation. Ghrelin, a hormone produced predominantly by the stomach, is the only known peripheral hormone stimulating food intake. Ghrelin promotes a state of positive energy balance, increases food intake and body weight and promotes accumulation of adipose tissue. Furthermore, ghrelin has been shown to exert anti-inflammatory effects, it reduces blood pressure and increases cardiac output. Therefore, ghrelin has been considered a potential anti-cachectic agent. We have designed and synthesized peptidic ghrelin agonists stabilized *via* amide bond formation between non-coded amino acids and fatty acid residues (12). Non-peptide ghrelin agonists and antagonists (CNRS, France) were also used in this study. Binding of ghrelin analogs to cells over-expressing ghrelin receptor and acute effect on food intake in mice, as well as their stability and pharmacokinetics was evaluated. The influence on food intake in acute cachexia

- lipopolysaccharide treated mice was studied (12). We aim to find the most promising ghrelin analogs with orexigenic and anti-cachexia action.

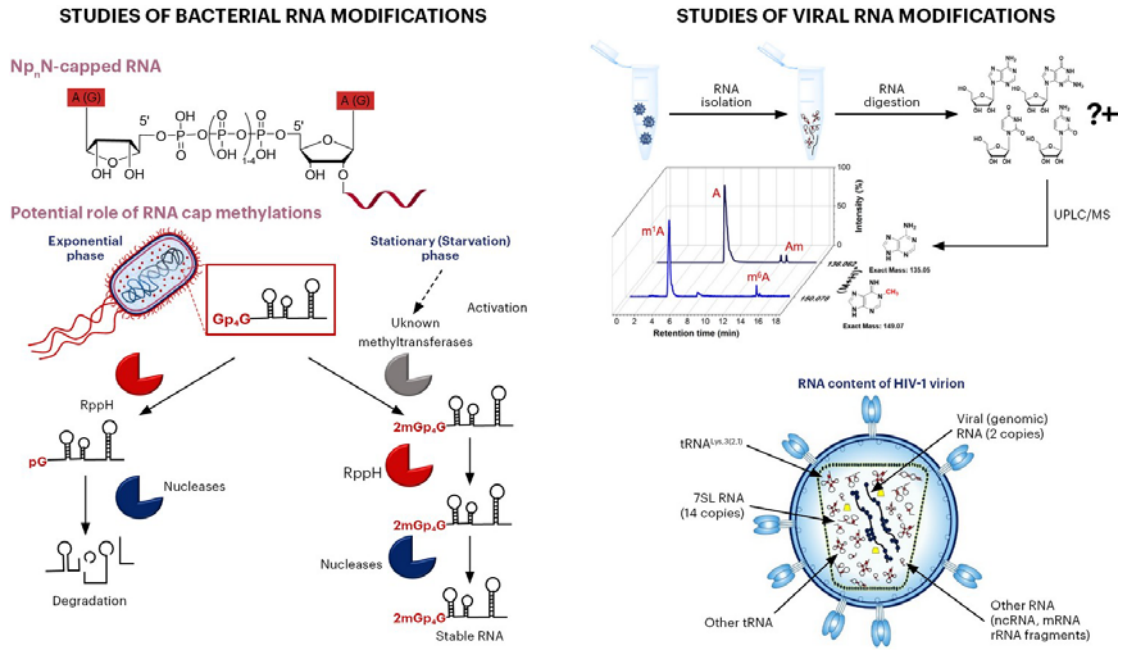
Selected key publications of the Maletínská group: (1) Maletínská L.et al. *Int J Obes* **2015**, 39, 986. (2) Prazienkova V. et al. *J Physiol Pharmacol*. **2016**, 67,121-8. (3) Holubová M. et al. *J Endocrinol.* **2016**, 229, 85. (4) Pražienková V. et al. *PLoS One* **2017**, 12, e0183449. (5) Mikulášková B. et al. *Nutr Diabetes* **2018**, 8, 5. (6) Čermáková M. et al., *J Proteome Res* **2019**, 18, 1735. (7) Špolcová A. et al. *J Alzheimers Dis* **2015**, 45, 823. (8) Popelová A. et al. *J Alzheimers Dis* **2018**, 62, 1725. (9) Holubová M. et al., *Neuropharmacology* **2019**,144, 377. (10) Strnad Š. et al. *Talanta* **2019**, 201, 364. (11) Pražienková V. et al. *J Alzheimers Dis* **2019**, 67, 1187. (12) Holubová M. et al. *J Pharmacol Exp Ther* **2018**, 366, 422.

The **Curtis group** is studying the functional potential of nucleic acids. One focus is on G-quadruplexes, which are four-stranded DNA and RNA structures stabilized by guanine tetrads. G-quadruplexes occur widely in nature, and are thought to perform a range of biological functions. The cellular machinery must have a way to distinguish G-quadruplexes in the genome with different functions, but the mechanism by which this is achieved is not well understood. Using a 496-member library made up of both canonical and noncanonical G-quadruplexes, we have shown that G-quadruplexes with different functions typically have sequence requirements that are overlapping but distinct (1-5). We recently obtained the first high-resolution structure of a G-quadruplex from our library (Volek et al., in revision). We are continuing this collaboration with the goal of obtaining addition structures, and also recently started a screen of our G-quadruplex library using proton NMR. These approaches should reveal a wealth of information about how different biochemical functions map onto G-quadruplex sequence space. In a second major area, we are using artificial evolution to identify DNA and RNA molecules with interesting and potentially useful functions. Topics of interest include developing new methods to generate signals using nucleic acids, identifying nucleic acid motifs that target double-stranded DNA in a sequence specific way, and identifying DNA and RNA motifs that covalently link themselves to viral proteins.

Selected key publications of the Curtis group: (1) Švehlová, K. et al. *Nucleic Acids Res* **2016**, *44*, 10789-10803. (2) Kolesnikova, S. et al. *Nucleic Acids Res* **2017**, *45*, 8684-8696. (3) Majerová, T. et al. *Biochemistry* **2018**, *57*, 4052-4062. (4) Kolesnikova, S. et al *ACS Chem Biol* **2019**, *14*, 1951-1963. (5) Kolesnikova, S. et al. *Molecules* **2019**, *24*, 3074.

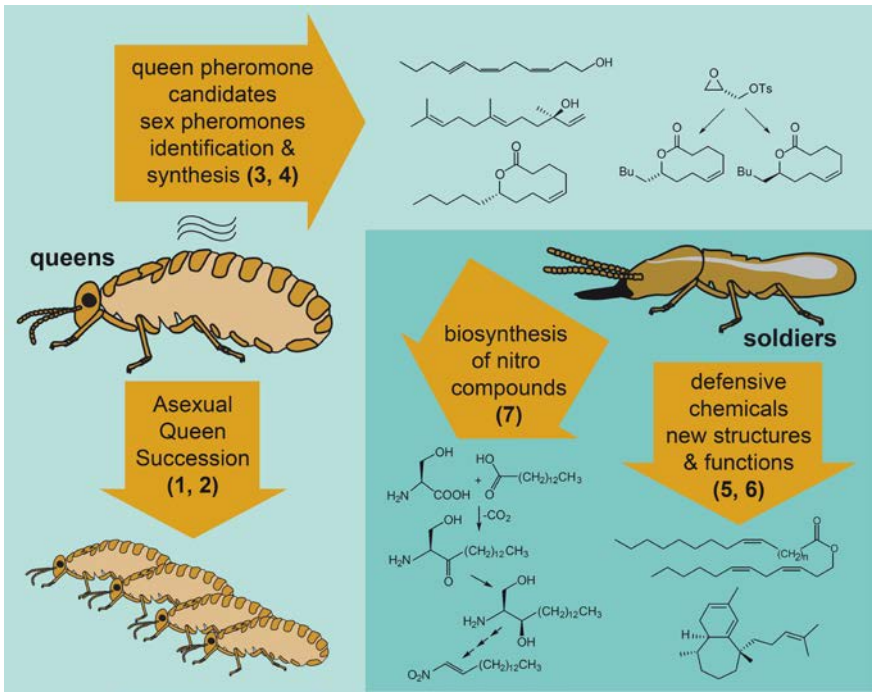
The **Cahová group** focuses on searching for new types of RNA modifications in viruses and bacteria and understanding their role. The group uses combination of various techniques ranging from LC-MS, various chemical biological and molecular biological methods to next generation sequencing (NGS). Studies of internal RNA modifications such as 1-methyladenosine (m^1A) in HIV-1 viral particles led to finding that the m^1A is a typical tRNA modifications and that the number of co-packed tRNAs in HIV-1 viral particle is 10-fold higher (1) than previously known. This work contributed to exclusion of theory that m^1A is another epitranscriptomic mark published in 2016. The second major topic of group was focused on searching for new RNA caps in model organism – *Escherichia coli*. Based on chemical similarity of dinucleoside polyphosphates (Np_nNs) with canonical eukaryotic cap, group proposed and confirmed existence of entirely new class of RNA caps in *E. coli*. The Cahová group identified nine new caps covalently attached to bacterial RNA based on unmethylated or methylated Np_nNs. These molecules in free form were known for more than fifty years and they were detected in almost all organisms. But Cahová group was the first to show that they can serve as RNA caps. Np_nNs can be co-transcriptionally incorporated into RNA by RNA polymerase and cleaved from RNA by at least two types of enzymes: RppH and ApaH. They propose mechanism of action of these caps. In the exponential phase of growth, bacteria continuously biosynthesize and degrade its own RNA to immediate react to environmental changes and the turn-over of macromolecules is high. The situation is different under starvation

conditions (represented by late stationary phase), when the cell does not have enough building blocks for making macromolecules such as protein or RNA. Thus, the bacteria start to incorporate and methylate Np_nNs caps in RNA. These caps are resistant to cleavage and RNA is stable. Cell cannot react flexibly to changes in the environment but it can survive harsh conditions with at least some “locked” RNA. After return to rich conditions, the methylated caps can be cleaved by newly expressed ApaH enzyme (2).



Selected key publications of the Cahová group: (1) Šimonová A. et al *Sci Rep* **2019**, 9**,** 8697, (2) Hudeček O., Benoni R. et al., *Nature Commun*, **2020,**11, 1052.

The **Hanus group,** in collaboration with Belgian and French partner laboratories, described a unique genetic organisation, dubbed Asexual Queen Succession, in colonies of several species of South-American higher termites. The queens of these species use a conventional sexual reproduction via eggs fertilized by the king to produce the sterile colony members and dispersing reproductives. By contrast, they use asexual reproduction (parthenogenesis) via unfertilized eggs, to produce harems of replacement queens. The resulting genetic structure of the colonies takes the best from both modes of reproduction: sexual process allows for high genetic diversity of sterile castes and dispersers, while the asexual process grants an undiluted genetic input of the founding queen into future queen generations (1, 2).



The group pursued its long-term activities in the field of chemical ecology of termites. Beside the descriptions of releaser pheromones (e.g. 3), the group focused on the quest for primer pheromones (queen pheromones), by which the present queens maintain their reproductive monopoly. The search for queen pheromone in several South-American higher termites species resulted in identification of queen-specific volatiles (e.g. 4), one of which was shown to really act as a queen pheromone (in prep.). In multiple tropical species, soldier-produced defensive compounds have been characterized, including identifications of new structures (5), some of which were shown to participate in intra- and interspecific communication (6). Biosynthetic pathway of soldier-produced nitro compounds has been elucidated in collaboration with the Max Planck Institute for Chemical Ecology (Jena), including the enzymatic network responsible for its key steps (7).
In the field of insect physiology, the group mapped the dynamics in endocrine regulatory pathways underlying the dramatic shifts in the physiology during the life cycle of bumblebee queens (8), and, in collaboration with the Biology Center (CAS), focused on the functional characterization of the juvenile hormone receptor in *Drosophila* (9).

Selected publications of the Hanus group: (1) Fougeyrollas, R. et al. *Proc R Soc B* **2015**, *282*, 20150260. (2) Fougeyrollas, R. et al., *Mol Ecol* **2017,** *26*, 3295-3308. (3) Dolejšová, K. et al. *J Chem Ecol* **2018**, *44*, 534-546. (4) Machara, A. et al. *J Nat Prod* **2018**, *81*, 2266-2274. (5) Kyjaková, P. et al. *Zool J Linn Soc* **2016**, *180*, 66-81. (6) Jirošová, A. et al. *J Chem Ecol* **2016**, *42*, 1070-1081. (7) Jirošová, A. et al. *Insect Biochem Mol Biol* **2017**, *82*, 52-61. (8) Jedlička, P. et al. Valterová, I. *Front Physiol* 7: 574. (9) Bittová, L. et al. *J Biol Chem* **2019**, *294*, 410-423.

The **Weiss group** is interested in voltage-gated-calcium channels, which are the primary mediators of the depolarization-induced calcium entry into neurons that initiates many cellular events. This group also studied involvement of calcium channels in human pathologies. (i) Clinical studies have suggested an increased prevalence for peripheral neuropathy in patients with pre-existing homocysteinemia. Their study provided the first experimental evidence that homocysteinemia is causally linked to the development of chronic pain by enhancing expression of Cav3.2 T-type calcium channels in nociceptive neurons via a PKC-dependent signalling pathway (1). (ii) Mutations in the gene Cacna1H encoding for Cav3.2 T-type channels are linked to the development of absence epilepsy. However, the mechanism by which these mutations enhance expression of the channel have remained unknown. This study

documented a previously unrecognized mechanism by which calnexin controls the trafficking of T-type channels traffic to the cell surface, and revealed that this regulation is disrupted by the GAERS mutations leading to an enhanced expression of the channel at the plasma membrane, providing the first pathological mechanism linking mutations in Cacna1H to absence seizures (2).

Selected publications of the Weiss group: (1) Gaifullina, A. et al. *Pain* **2019**, 160, 2798. (2) Proft, J. et al. *Sci Rep* **2017**, 7, 11513.

The **Kečkéšová group** Despite considerable effort in the last decades to characterize and understand the vulnerabilities of cancer cells, many important aspects of cancer biology are still poorly understood, and are delaying the implementation of effective cancer treatments. While cancer can affect many different parts of the body with high frequencies, some types of cancer are extremely rare. This point to the possibility that some tissue-types, and/or specific subset of cells within some tissues have already devised ways of countering cancer formation, and as such, could provide scientists with novel insights into the prevention and/or treatment of cancer and can serve as a great source for uncovering new tumour suppressor pathways. Research in this area by Dr. Kečkéšová led to a creation of a list of 87 genes with increased probability of being effective tumour suppressors. One of these genes, called LACTB (Lactamase B-like), was characterized further in more detail, and revealed that LACTB is a novel and highly potent mitochondrial tumour suppressor. It acts through reprogramming of cancer lipid metabolism what leads to differentiation of cancer stem cells (Keckesova et al., Nature, 2017). This study opened new avenues for research and created many exciting questions and possibilities that the Kečkéšová group is researching. Firstly, they aim to characterize the LACTB tumour suppressor pathway in more detail, uncover the unknown aspects of its mechanism (such as the physiological regulation of LACTB, identity of LACTB substrate, and the role of metabolism in the differentiation program of cancer cells) and to examine the therapeutic potential of this pathway. Secondly, they will test other proteins from the 87 signature list for their tumour suppressive properties and identify novel tumor suppressor pathways in human cancer. Completion of these two aims will reveal novel cell-cycle, cell-state and cell-energy regulatory mechanisms in human cancer cells that might direct us to more effective cancer treatments. The group was established relatively recently, in 2018, and the first results obtained in IOCB should be published during 2021.

The **Vondrášek group** major achievements were realized in 3 interconnected areas primarily focused on a general phenomenon – interactions of biomolecules. These areas span range of interaction types, from protein-protein to protein-ligand and DNA ligand interactions. Special attention was also paid to influence of post-translational modifications on interaction properties of biomolecules.
It is possible to characterize interactions of proteins with DNA experimentally as well as theoretically. We explored theoretical ways how to realistically model interactions with experimentally determined Kd and very good agreement between theory and experiment was achieved (1, 2). MD simulations were used in different setups to show how the current approaches and methods quantitatively describe the process and what the conditions of their utilization are. Post-translational modifications – namely phosphorylation - are responsible for transition of conformations towards states which determine interaction properties. In paper by Vymetal et al (3) the influence of phosphorylation on local conformation states was described and differences initiating in application of different force fields were discussed.
Large effort of the group was devoted to development of tools useful in molecular and chemical biology. There were 3 tools developed in the group. First, interoperable chemical structure service making possible to link chemical resources with molecular biology resources (4). The second service, which was created in collaboration with EMBL-EBI, offers visualization of residues conservation in proteins families and their structural importance (5). Finally, online server for analysis of proteins structures and their stability offers various ways for

characterization of not only protein stability but also protein-protein and protein-DNA interactions (6).

Selected publications of the Vondrášek: (1) Jakubec, D. Vondrášek, J. *J Chem Theory Comput* **2019**, 15, 2635. (2) Stasyuk OA. et al. *J Chem Theory Comput* **2017,** 13, 877. (3) Vymětal, J. et al. *J Chem Theory Comput* **2019**, 15, 665. (4) Kratochvíl, M. et al. *J. Cheminformatics* **2019** (11) , article number 45. (5) Jakubec, D. et al. *Bioinformatics*. **2019**, 35, 332. (6) Galgonek, J. et al. *Nucleic Acids Research*. **2017,** 45, W388.