

Description of the main research directions investigated by the institute

The main research directions are (i) light-responsive inorganic molecules and materials, (ii) chemistry of boron compounds, (iii) new materials for a sustainable environment, (iv) conservation and cultural heritage science, and (v) geochemical analysis of sediments. Applied research then forms an irreplaceable part of activities in all these directions. The fulfilment of the research program is discussed by the Institution's board at the end of each year. The research is carried out in two main departments of the Institute, two interdisciplinary laboratories and in particular tasks it is supported by the activities of the service centre of instrumental techniques. At the same time, it is not uncommon for research teams of individual departments to work closely together in solving a number of tasks, which leads to greater synergy and integrity of research activities in the Institute.

1. Light-responsive molecules and materials

Key persons: Kamil Lang, Michael Londesborough, Kaplan Kirakci, Jan Demel

This research domain seeks to discover and develop novel molecules, nanostructured materials, and (nano)materials with inherent luminescent (fluorescence, phosphorescence, oxygen- or temperature-dependent luminescence) and photosensitizing properties (singlet oxygen, $O_2(^1\Delta_g)$, production). The focus is on systems that satisfy critical parameters such as stability in water, photostability, transparency, and non-toxicity, and that display useful interaction with surrounding components, gas or ion diffusivity, and bio-compatibility. The main targets are sensitizers for photodynamic therapy (PDT), radiosensitizers for X-ray induced photodynamic therapy (X-PDT), and photoantimicrobials. This topic has been developed over a longer period mainly at the Department of Materials Chemistry under the leadership of the current director of the Institute, Kamil Lang. Recently, however, the team of Michael Londesborough in the Department of Synthesis has made significant contributions to this research topic by examining the photophysical properties of the binary borane *anti*- $B_{18}H_{22}$, the centrosymmetric isomer of octadecaborane, and a number of its derivatives. It represents a nice example of mutual interaction and thematic connection between individual working groups in the IIC. The main results of this research direction achieved in the evaluated period can be summarized as follows.

Transition metal cluster complexes. The IIC scientists, particularly Kamil Lang and Kaplan Kirakci, synthesized and characterized a number of new octahedral molybdenum cluster complexes (Mo_6) and were the first to propose that certain transition metal cluster complexes can act as versatile theranostic tools with desirable features such as high luminescence quantum yields, production of singlet oxygen, and high radio-opacity, thus making these clusters useful as contrast agents, and radioluminescent properties attractive for X-ray luminescence computed tomography or X-PDT of cancer. In this respect, we propose to synthetically tune these cluster complex biomaterials for efficient *in-vitro* and *in-vivo* singlet oxygen photosensitization and radiosensitization with a special attention to specific cancer cell targeting. We envisage a promising future for these materials in antimicrobial coatings, for use in photodynamic therapy, and in oxygen sensing, due to their high photostability that enables their application over long periods and gives efficient production of cytotoxic singlet oxygen.

It was found that (i) the inorganic core of Mo_6 complexes is responsible for broad absorption and red luminescence bands, long-lived triplet states and high luminescence quantum yields both in the absence of oxygen, (ii) the triplet states, formed upon excitation with UV, blue light or X-rays, are efficiently quenched by oxygen leading to the formation of $O_2(^1\Delta_g)$ in high yields, and (iii) the deliberate selection of the apical ligands allows for an optimization of the solubility and chemical stability especially in aqueous media which is primordial for biological applications. Strategies towards stabilization and optimization of properties of Mo_6 complexes for photodynamic applications have been delineated. It was documented that

especially some Mo_6 complexes are applicable as photosensitizers in PDT and radiosensitizers in X-PDT. Experiments *in vivo* are under way. The portfolio of investigated compounds also involved copper-, tungsten- and rhenium-based cluster complexes.

Organized assemblies of porphyrin units belonging to the family of porphyrin-based covalent organic frameworks and metal-organic frameworks. Porphyrin units, which have the ability to produce singlet oxygen upon visible light irradiation, serve as building blocks for biomaterials with novel functional entities designed to enhance specific photophysical and photochemical properties. By varying the connecting moieties control can be imparted on the surroundings of the porphyrin units and prevent their close contacts, i.e., providing structural control that, in turn, enables the tailoring for photophysical applications. These materials are being developed for application in areas such as photodynamic antimicrobial coatings, PDT (especially in the form of nanoparticles), and site-specific oxygen sensing.

In the evaluated period, physico-chemical, photophysical, photochemical and photobiological properties of new porphyrinoids and porphyrin-based (nano)materials have been systematically investigated, particularly it included covalent organic frameworks (COFs), conjugated microporous polymers (CMPs), metal-organic frameworks (MOFs), nanoparticles and polymeric nanoparticles and nanofibers. Especially, the photosensitized inactivation of pathogens has become an attractive strategy to combat drug-resistant microbes or viruses. Its advantages encompass a broad spectrum of action, limited damage to the host tissue, and high efficacy, i.e., singlet oxygen attacks several critical components of bacteria and viruses, thus making their resistance to singlet oxygen unlikely.

Luminescent borane molecules. Of all the known binary boranes (compounds consisting only of boron and hydrogen), *anti*- $\text{B}_{18}\text{H}_{22}$ is the only one to have the property of inherent fluorescence. In 2015, it was discovered by Michael Londesborough, that solutions of *anti*- $\text{B}_{18}\text{H}_{22}$ are capable of laser emission with a good efficiency and an outstanding resistance to photodegradation when compared to commercial organic laser dyes; making it the first laser borane. The new borane laser has a resistance against degradation that is superior to many of the commercially available state-of-the-art blue laser dyes. This means that the number of times the liquid medium has to be replaced is reduced, helping to solve issues with costs, occupational hazards and environmental impact due to the handling of solvents that are often toxic and flammable. In addition, the excellent solubility of *anti*- $\text{B}_{18}\text{H}_{22}$ in organic environments enables its incorporation into polystyrene polymer matrices without the loss of its fluorescence properties. The resultant new range of *anti*- $\text{B}_{18}\text{H}_{22}$ /polymer nanocomposite materials with interesting optical properties have potential for use as coherent light sources in optoelectronic, spectroscopic and sensing devices, and as chromophores in luminescent solar concentrators.

Discovery of new borane laser has been published in Nature Communications in 2015. The paper significantly contributed to the exposure of the Institute to the general public via reference in several popular science publications, international magazines on optics, and Czech national newspapers. Current research is expanding on these promising beginnings, seeking to fully understand the potential and limitations of this and other new luminescent borane molecules, and novel borane nanoparticles, at a fundamental level with an aim to maximise laser efficiency and photostability, and offer emission at tuneable wavelengths. If *anti*- $\text{B}_{18}\text{H}_{22}$ is combined with unusually structured new boron hydride, $\text{B}_{18}\text{H}_{20}(\text{NC}_5\text{H}_5)_2$, which luminescence is being linked to the flapping-like motion of its pyridine ligand “wings”, wide-range absorption of light (across more than 300 nm) and energy concentration is achievable. This significant spectral coverage is possibly useful for luminescent solar concentrators – devices that collect light energy over a wide range of frequencies and concentrates that energy into a single wavelength emission.

2. Chemistry of boron compounds

Key persons: Bohumír Grüner, Bohumil Štíbr (†2020), Drahomír Hnyk, Michael Londesborough, Tomáš Baše, Oleg Tok

The study of boron hydride chemistry belongs to a core competency of the IIC. The IIC boron group has undergone a diversification from its traditional focus on new types of boron hydride clusters to include a spectrum of potential outlets for the chemistry, such as in biomedicine, surface chemistry, weakly coordinating anions, extraction agents for radionuclides and optics. Novel topics have been introduced by B. Grüner, current Head of the Department of Synthesis and later by key members of the team D. Hnyk, M.G.S. Londesborough, T. Baše and O. Tok. In the evaluated period, one of the founding and life-long active members of this group, Bohumil Štíbr also actively participated in the research. Unfortunately, in January 2020 he passed away. Therefore, his last recognition, the most prestigious Czech Academy of Sciences medal, „*De scientia et humanitate optime meritis*“, planned to be awarded to him on the occasion of his 80th's birthday, will unfortunately not reach him alive. A special issue of *Molecules*, devoted to boron cluster chemistry, was organized in his memory. Boron hydride chemistry at the Institute can be divided to the following research areas: (i) systematic synthetic chemistry of carboranes, metallacarboranes and main group heterocycles, (ii) biologically active compounds, (iii) luminescent boranes (already described in the previous chapter), (iv) boron cluster molecules for self-organized nanosystems, and (v) theoretical chemistry of polyhedral boron compounds.

Systematic synthetic chemistry of carboranes, metallacarboranes and main group heterocycles. During the five years' evaluation period one of our main focuses has been on the basic chemistry of boron cages, particularly the chemistry of tricarbollides, ten-vertex dicarbaboranes and aspects of their substitution reactions. Several key findings have been made. For example, when reacting *arachno*-6,9-C₂B₈H₁₄ with acyl chlorides ROCl, the product strongly depended on the nature of R. The majority of acyl chlorides gave 8-R-*nido*-7,8,9-C₃B₈H₁₁ species, but bulky acyl chlorides (*adm*, *mes*, *naph*, *anth*) induced unusual cage rearrangements to give derivatives of *closo*-1,6-C₂B₈H₁₀. The explanation of such a stereospecificity that dictates these reaction pathways belongs amongst the greatest achievements of Bohumil Štíbr (†). Electrophilic halogenation and methylation of the ten-vertex *closo* dicarbaboranes were also investigated. The permethylated compounds proved to be extremely air stable in comparison to their unprotected counterparts. We think this to be a consequence of their rigid, egg shaped hydrocarbon structures incorporating inner C₂B₈ carborane scaffolding. Such findings and the development of basic synthetic methodology provides a basis for the more targeted areas of our research program. For example, in the design, synthesis and modification of C-substituted carboranes and the cobalt bis(dicarbollide) anion, which have become increasingly important structural motifs for the design of new biologically active compounds and materials.

Building on our discovery in 2013 that we published in *Angew. Chem.*, that sulfamide carboranes can act as a promising class of specific Carbonic Anhydrase-IX (CA IX) enzyme inhibitors, we have synthesized and characterized a series of cobalt bis(dicarbollide) species that show highly specific and selective inhibition of the CA-IX isoenzyme that is crucial in several oncological diseases. This finding is one of the most important in Bohumil Grüner's research, which generally focuses on biologically active boranes and medical research. Similar synthetic techniques to substitution of boron clusters have been applied by Michael Londesborough to the highly photoactive compound *anti*-B₁₈H₂₂, enabling an explosion of new derivatives. Thus, halogen, pyridine, thiol and alkyl derivatives of this boron hydride have been made, and their chemical and photophysical properties fully delineated, producing a portfolio of novel luminescent borane species. With regards to novel syntheses, we have discovered recently a new family of heterohelicenes built from fused siloles with up to 20-30 condensed heterocycles, the longest helicene known so far. This new area of silicon chemistry, introduced at the IIC by Oleg Tok, is developing further to include new types of saturated and unsaturated (poly)heterocyclic compounds containing B, Si, Ge, Sn, O, S, and N in various combinations. These compounds are potentially useful in molecular electronics, capacitors and Si-C composites.

Biologically active boron compounds. Carbonic Anhydrase-IX (CA-IX) is a target enzyme for the anti-metastatic treatment of hypoxic tumors. New generations of polyhedral inhibitors of CA-IX, based on the selection of boron cages, optimized substitutions and their stereochemistry, have been developed at the IIC. The structure-activity relationship (SAR) has been examined using a library comprising over 70 new polyhedral inhibitors of CA-IX prepared and tested within evaluated period (ca. 85 in total). These results were complemented by approximately 48 synchrotron structures of enzyme-inhibitor complexes that covered all promising types of inhibitors, and by theoretical and physicochemical studies. The structural studies provided rationale for enhanced activity of the new families of compounds. In addition, pharmacologically relevant factors such as plasma protein binding, cell membrane penetration, toxicology and pharmacokinetic studies (mice model) were performed within external collaboration with Institute of Molecular and Translation Medicine at Palacký University, Olomouc, Czech Republic (IMTM) on a panel of the selected inhibitors. The tests have shown significant reduction of tumor size shown on two cancer lines when the animals were treated by selected members from both carborane and metallacarborane families. Due to promising properties, these compounds are considered as good candidates for drug development applicable in cancer treatment. Nevertheless, due to their high selectivity for tumor-associated transmembrane CA-IX protein, these boron species may also offer a reasonable potential for future use in imaging methods and Boron Neutron Capture Therapy (BNCT) treatment.

Boron cluster molecules for self-organized nanosystems. This research theme targets mainly two-dimensional surface-supported and self-organized structures and patterns of borane and carborane constituents. These constituents of 2-dimensional self-assembled monolayers exist in a number of isomeric forms with practically identical geometry but different physical properties such as dipole moment magnitude and orientation. Furthermore, they exhibit high chemical and thermal stability, and, due to the low conformational freedom of their relatively rigid skeletal structures, they show a lower total number and fewer types of defects in their 2-D assemblies. In the period of 2015-2019 several studies have been concluded in the Institute in order to shed more light on aspects such as the interaction between these molecules' anchoring thiol groups and a flat gold surface revealing clearly the existence of two binding modes associated with their inherent SH bond scission aptitudes. A very good understanding of how carborane-dithiols interact *via* their SH groups with a gold surface has been achieved. There is a simple acid-base chemistry to control the way these two isomeric species are adsorbed onto a surface. The poetic expression, describing the modes of carborane-thiol linkages, of "molecules doing the can-can" comes from how they "stand on" either one or both anchoring groups, the other one being lifted up (i.e., physisorbed). As an award for scientific work in this field of research Tomáš Baše received prestigious Fulbright-Masaryk support for his stay at UCLA in 2019.

There are many other examples showing that self-assembled monolayers, their structure, stabilities and properties are the result of a whole pack of interactions. Special attention is recently paid to the translation of single constituent inherent properties to collective properties of the respective two-dimensional assemblies, and thus to better understanding of self-assembly principles and driving forces. The obtained knowledge is going to be applied to building blocks design towards functional nanosystems applicable in areas that range from electronic devices, molecular recognition systems, and anti-corrosive surface treatment to sensing and responsive materials in biology.

Theoretical chemistry of polyhedral boron compounds. The main aim is the analysis of electronic structure in boron clusters and the prediction of molecular sites for possible substitutions. Further theoretical and experimental investigations of chalcogen and pnictogen bonding are also included, mainly in relation to the mechanism of inhibition of various enzymes. Paramagnetic ^{11}B NMR spectroscopic parameters of metal-containing borane clusters is being evaluated at the high computational level. Two of the more important achievements obtained in evaluated period by Drahomír Hnyk are: Firstly, progress has been made in understanding further types of σ -hole interactions that are responsible for crystal

packing forces in heteroboranes. For example, a novel stibacarbaborane, i.e., nido-7,8,9,11-Sb₂C₂B₇H₉ was prepared and in light of the computation of the electrostatic potential (ESP) a unique σ -hole was found in the middle of the Sb-Sb vector and was characterized by so far the most positive value of ESP, 42.7 kcal.mol⁻¹ in the large body of heteroboranes and a very strong pnictogen bonding in the corresponding crystal was thus revealed. Secondly, the multidimensional statistical method known as factor analysis was applied to a series of BX₃ compounds that are experimentally available. Their experimental ¹¹B NMR spectra agree well with the computed data and this statistical approach provided a deeper insight into the nature of the magnetic shieldings in this series, the magnitude of the ESP (related to π -hole magnitude) on the central boron atom being one of them.

3. New materials for a sustainable environment

Key persons: Jiří Henych, Jan Demel, Václav Štengl (†2018), Petra Ecorchard, Jan Šubrt

The growing global population is dramatically increasing the level of pollution in all components of our environment; the atmosphere, waterways and soils. This research activity focuses on both the development of new materials and the optimization of the properties of known materials for environmental applications and use under real conditions.

Photocatalytic materials. Although many new catalysts for the photodegradation of pollutants have been introduced in the last decades, including composite materials with graphene, nanodiamonds, carbonaceous or other quantum dots, nanostructured TiO₂ remains the most promising material for photocatalytic applications in the environment. Research into TiO₂ materials has a very long tradition at the IIC, connected mainly with the work of Jan Šubrt and Václav Štengl (†) and their groups. Current progress is being made through the joint efforts of the Department of Materials Chemistry and the Centre of Instrumental Techniques, looking to optimize the excellent photocatalytic activity of TiO₂ and its various morphologies by controlling the particle size. In addition, we have made and studied TiO₂ particles with surface and bulk modifications with a large range of metals and heteroelements. A number of composites have also been prepared (e.g., with SiO₂) for use in self-cleaning paints, screeds for concrete surfaces or wood preservatives in order to protect constructions against bio-degradation. Further, an amorphous form of titanium dioxide was intensively studied for its excellent sorption activity, especially regarding the removal of heavy metals and radionuclides from an aqueous environment. Currently, we are developing a "floating photocatalyst" that combines the sorption and photocatalytic properties that we have delineated. This material could be used to purify surface waters from low concentrations of stable aromatic pollutants, including biphenyls and dioxins.

Reactive sorbents. Reactive sorbents with high activity towards organophosphorus pesticides and chemical warfare agents (CWAs) have been developed in the Department of Materials Chemistry under leadership of Václav Štengl (†), now Jiří Henych. These materials are useful for protection and decontamination purposes in military and civilian areas. The stability of sorbents in various matrices, solvents, or thin films is crucial for practical applications. Besides the most studied titanium dioxide with photocatalytic properties, the research effort was also focused on the preparation of nanostructured metal (hydr)oxides with attractive surface properties such as CeO₂, MnO₂, Mg(OH)₂, FeOOH₆ or CuO. As an example, Sn-doped CuO nanosheets were synthesized by a solution method and the influence of tin doping on the morphology and catalytic decomposition of a pollutant model was described in detail. Besides doped single metal oxides and metal oxide/carbon composites, mixed metal oxides and their composites also showed a good catalytic activity. Water-based procedures to prepare composites TiO₂/CeO₂, TiO₂/iron oxides and mixed oxides/clay minerals have been developed, where the synergistic effects enhance pollutant decompositions (organophosphorus pesticides, azo dyes, drugs, CWAs and their simulants). Sonochemical syntheses of low-dimensional materials has been developed and optimized - it includes the preparation of graphene oxide (GO) and its use for the construction of highly active TiO₂/GO composites in degradation of toxic compounds. Furthermore, the

sonochemical approach for deaggregation of nanodiamonds (so called salt-assisted ultrasonic deaggregation) has been introduced.

Layered materials. This research is aiming at the production of 2D materials such as graphene, its inorganic analogues and double-layered hydroxides (LDH) stabilized in various solvents and matrices and the fabrication of new graphene-polymer composites with applicable mechanical or electronic properties. In addition, graphene-based composites with a high sorption capacity for radionuclides (^{137}Cs , ^{85}Sr , etc.) are being fabricated and investigated. As one of innovative approaches introduced by Petra Ecorchard – head of the Centre of Instrumental Techniques, 2D materials were modified with ionic liquids whose potential is, for example, to improve the mechanical and thermal properties of polymers. The goal of modification by ionic liquid was to develop a system that will stabilize the 2D material from the agglomeration.

Porous coordination polymers. Metal-organic frameworks (MOFs) belong to the fast-growing area of organic-inorganic coordination polymers. The structure of MOFs is combining metal nodes and polytopic organic ligands (linkers). The high surface area of MOFs, along with the possibility of introducing functional groups, make them highly promising materials for many applications, including sorption and degradation of pollutants. One of the drawbacks of many MOFs is their low stability in water that comes from the lability of the coordination bonds towards hydrolysis. For this reason, the main attention was paid to (i) characterization of the stability and degradation mechanisms of Zr-based MOFs (and (ii) the preparation of novel MOFs with high hydrothermal stability. Further, a new family of MOFs based on phosphinate linkers have been developed by Jan Demel at the Department of Materials Chemistry. It was demonstrated that the isorecticular architecture, well known from carboxylate MOFs, is applicable also for phosphinate MOFs, which is in contrast to phosphonate-based MOFs. Thus, the combination of Fe^{3+} ions with the bisphosphinate linker leads to a new MOF, named Fe-ICR-2 (ICR stands for Inorganic Chemistry Rez), with a honeycomb structure. Importantly, Fe-ICR-2 is endowed with higher hydrothermal stability in comparison with a carboxylate-based analogue Fe-MIL-53.

4. Conservation and cultural heritage science

Key persons: David Hradil, Silvie Švarcová, Petr Bezdička

The preservation and interpretation of European cultural heritage is an important challenge relevant to modern society. Considering this high societal impact, a joint workplace of the IIC with the Academy of Fine Arts in Prague has been established already in 2004. The ALMA laboratory (Academic Materials Research Laboratory of Painted Artworks) performs interdisciplinary research that focuses on the application of inorganic chemistry to the advanced materials microanalysis of objects of art and the study of chemical degradation processes of pigments in paints. The results from these studies help in the subsequent evaluation of works of art in terms of their originality, age, provenance, and in the implementation of innovative conservation methods. Our systematic approach to research includes, in particular, the adaptation and testing of micro-analytical methods for non-destructive investigation of paint micro-samples and implementation of non-invasive approaches to the fine arts analysis and imaging, which are provided either in-situ (with portable instrumentation) or ex-situ (with large-area scanners or customized laboratory equipment). Despite the predominant analytical nature of the laboratory, many activities show significant interdisciplinary overlaps - towards geosciences (in the study of provenances of mineral pigments), chemical syntheses (in the preparation of reference substances, replication of pigment production etc.) or towards the history of art (in interpreting painting techniques).

Microanalysis of pigments. ALMA laboratory is unusual in its emphasis on mineralogical analysis and the application of geosciences to the research of fine arts. These approaches allow to differentiate inorganic pigments more finely - not only by their type, but also by their structural varieties, the methods of their artificial production, or according to the place of

extraction of the natural raw material. In some cases, e.g., Baroque clay-based grounds, the origin of the raw material determines also the regional provenance of the painting. Microanalysis of clay minerals in the preparatory and painting layers, especially by the method of laboratory powder X-ray micro-diffraction, is one of the pioneering topics systematically developed by David Hradil. In general, tracking and linking the paths of the materials and technologies with the paths of the artists is very challenging but we have achieved success here through our systematic approach. It was found, for example, that the clays used in some northern Italian paintings of the 16th to 17th centuries have been identified as identical to those previously used by Renaissance masters to create terracotta sculptures. The composition of the grounds is so specific that it is possible to distinguish neighbouring regions - works that Carravaggio and others painted in Italy and in Malta.

Degradation and alteration processes. Degradation processes are another important direction of recent cultural heritage research. The increased interest in the understanding of the various chemical processes in the colour layer of pieces of art was caused by numerous misinterpretations (iconographic, historical) that did not take into account secondary visual changes in an artwork. Within the evaluated period, the processes causing colour changes of pigments as well as those processes leading to the loss of cohesion of paints have been experimentally investigated. Within these investigations, one finding of particular note, which we were able to describe, are the complex formation of metal soaps, their crystallization, aggregation and migration towards the painting surface. Significant achievements have been made in the research of Silvie Švarcová, who focused on the description of the crystal structure of neo-formed Pb-carboxylates. It was found that both C16 and C18 chains are present in one crystal structure, creating the statistical disorder at the ethyl end of the chains. ²⁰⁷Pb ssNMR spectroscopic study of these species revealed two distinct local environments of lead atoms, corresponding to the symmetrically and asymmetrically substituted lead carboxylates; the formation of a holo-directed structure for both the structural motifs has thus been confirmed. The detailed differentiation of metal soaps' structures will lead to a better understanding of the causes of their formation.

In ALMA, the testing, optimization and use of powder X-ray diffraction (pXRD) has a long tradition thanks to the expertise of Petr Bezdička. ALMA has played a pioneering role in the use of powder X-ray micro-diffraction (micro-pXRD) in the field of cultural heritage research, showing its effectiveness in the analysis of heterogeneous fragments of paints or their cross-sections without any pre-treatment. The field of application of micro-pXRD is very wide – it has an irreplaceable role in the provenance analysis of mineral pigments, as well as in the analysis of degradation products. Recently, the method has also been adapted for the measurement of small paintings (miniatures), which is a completely new example of its application.

5. Geochemical analysis of sediments

Key persons: Tomáš Matys Grygar, Martin Faměra

The research topic of geochemical analysis was born inside the former analytical laboratory of the IIC. Thanks to the systematic work of Tomáš Matys Grygar, it gradually became independent and established itself as a new research direction. As a result, the Laboratory of Environmental Geochemical Analysis (LEGA) was created and the number of its national and foreign collaborations is gradually growing. Close cooperation with Faculty of Environment, J.E. Purkyně University in Ústí nad Labem (UJEP), which generates students and new employees participating in LEGA projects, is especially important for the team's personnel growth. The team is focused on the integration of chemistry to a holistic approach on the research of sediments and sedimentary environments, with a particular focus on the understanding of paleoenvironments. A key topic is a study of the spatial distribution of contaminants in floodplain internal sedimentary structure (floodplain architecture), in particular risk elements. Here, chemical processes are particularly important to the post-depositional fates of the contaminants. The second core topic is a more general sediment

geochemistry analysis focused on paleoenvironmental reconstructions from sediment composition.

Studies on floodplain architecture. The impact of human beings on floodplain architecture (including floodplain aggradation) is commonly overestimated, and floodplain sediment reworking is correspondingly underestimated. It was revealed, e.g., in the research of Martin Faměra et al., that lateral deposition is more relevant than aggradation, if the area is impacted by catchment deforestation, ore mining, and failures of settling pond. The paradigm of human-enhanced sediment accumulation in floodplains triggered by tillage and land cover changes in catchments should be reconsidered and revisited in better planned future case studies. Further, it was found, that numerous current methods and routines in fluvial contamination studies are principally incorrect and sampling protocols must be change to avoid erroneous understanding of floodplain architecture. They must consider sediment recycling and the high variance in age of sediments. In numerous case studies, the usefulness of an integrated approach to floodplain architectural studies was shown. Contaminated floodplains usually have contamination focused in spatially limited hotspots (transversal heterogeneity) and in specific segments along the watercourse (longitudinal heterogeneity). Historical contamination events, such as radioactive floods with ^{226}Ra and U peaks in the catchment areas of the Ohře River and the Ploučnice River or historical mining with a contamination peak in the 16th century, such as Sn mining in the Ohře River catchment area (produced “cheap” and easily accessible isochronous surfaces in floodplain sediment bodies, valuable in studies of floodplain genesis).

Sediment geochemistry and paleoenvironmental reconstructions. Chemical weathering indices are broadly used in paleoclimate reconstructions based on correct chemical compositional data analysis of sediment (CoDA). The major achievements of Tomáš Matys Grygar and his team in this subtopic stem from a ten years' study of the Most Basin, Ohře Rift, NW Czech Republic, with several hundred metres of lacustrine sediments taken from ca. 20 drill cores in the last decade. It was shown that the Most Basin hosts the most detailed (continental) archive covering nearly 2 My from around the beginning of the Miocene climatic optimum (around 17.0 Ma); sediments were dated using a state-of-the-art integrated approach of magnetic polarity and cyclostratigraphy. Due to the absence of fossils and biogenic sediment components, the entirety of useful information from the Most Basin deposits was derived from the chemical composition of its siliciclastic detritic components. The work on weathering indices in LEGA is continuing and has already brought novel weathering indices and novel statistical tools in CoDA for paleoclimatic research, which were published in top-ranked journals. The sediment geochemistry logs can be interpreted in terms of climate (or orbital signatures) only if these logs are laterally stable, independent on sediment lithology, and show strong and unequivocal Earth orbital signatures.

6. Applied research

Key persons: Zbyněk Černý, Jan Šubrt, Petra Ecorchard, Jiří Henych, Bohumír Grüner

In order to utilize the potential of the compounds and materials developed within the IIC, systematic efforts are devoted to finding possibilities for their commercial use, particularly in fields of (i) the building industry, (ii) nuclear safety, (iii) 3D printing (iv) water treatment, and (v) remediation of toxic compounds. Several areas of use, including medicine, are covered by (vi) new boron compounds.

Building industry. This field of application includes the development of remedial products, which are either zinc-based nanosols (developed in the group of Zbyněk Černý) or photocatalytic TiO_2 -based composites for self-cleaning surfaces (developed in the Centre of Instrumental Techniques), for plaster and facades suffering with green algae and mould infestation. In addition to nanocomposite coating systems for the surface protection of historical buildings and other heritage objects, photocatalytic active screed on cement-based

application especially on concrete surfaces and the photoactive powder ingredient in concrete have also been developed.

Nuclear safety. This field of application includes the development of a new generation of non-flammable inorganic materials with high contents of neutron absorbents that can be used for shielding neutron and gamma radiation (Zbyněk Černý) as well as highly active absorbents based on hydrated titanium oxide in the form of needle aggregates of nanoparticles that are designed for use in sorption of radionuclides from contaminated waters and their separation from aquatic environment (Jan Šubrt, Petra Ecorchard).

3D printing. A new technology of 3D printing, based on original ceramic-like inorganic systems and artificial sandstones, has been introduced in the group of Zbyněk Černý capable of printing artefacts for art design. These new systems are highly tunable in their formulation, non-flammable, non-toxic, stable under outdoor exposures, environment-friendly and reasonably priced, which is very important factor.

Water treatment. With the changing climate, the issue of effective water management in the Czech Republic is a clear national priority. Regarding the safe utilisation of drinking or process water (rainwater, recycled water), its stability and quality can be improved by means of products containing nanoparticles of silver (Ag). An original method for preparing selected substrates with Ag nanoparticles fixed to their surfaces has been developed at the IIC (Zbyněk Černý). These substrates, depending on the method and type of application, can be either synthetic or natural carriers with particle size ranging from microcrystalline powders to rough particles. The high-surface area substrates, mainly in combination with photocatalytic TiO_2 , are suitable for the preparation of original porous coatings that offer a variety of technical solutions to flow water treatments.

Remediation of toxic compounds. The group of Jiří Henych had developed unique methods for preparing powdered nanostructured oxides of various metals that quickly and effectively bind organophosphorous-based neural paralysing poisons (e.g., Sarin, Soman, VX and organophosphorous pesticides) to their surfaces and then chemically inactivate them; these materials also work with other toxic materials, such as, e.g., yperite. At the moment the new sorbents are being tested and compared with the currently available commercial materials that are used as means of protection by the military all over the world. The preliminary results show that the efficiency of new materials prepared is superior.

New boron compounds. In the group of Bohumír Grüner new methods for the production of $\text{B}_{10}\text{H}_{10}^{2-}$ and anhydrous $\text{M}_2\text{B}_{12}\text{H}_{12}^{2-}$ or $\text{MB}_{12}\text{H}_{12}$ ($\text{M} = \text{Li}^+$, etc.) have been recently introduced. These technologies will be subject of larger scale verifications and licensing. Salts of $\text{B}_{10}\text{H}_{10}^{2-}$ and $\text{B}_{12}\text{H}_{12}^{2-}$ ions and their derivatives are applied as solid electrolytes for batteries, in drug design, boron neutron capture therapy, catalysis, and in automotive industry and thus the developed synthesis procedures could find practical applications.

Research activity and characterisation of the main scientific results

1. Chemistry of boron compounds directed to medicinal research

Previous to this evaluation period, in 2013, we described that sulfamide carboranes can act as a promising class of specific Carbonic Anhydrase IX (CA IX) enzyme inhibitors (*Angew. Chem., Intl. Ed. Eng.*, 2013, 52, 13760-13763). This isoenzyme is associated with solid hypoxic tumours and belongs to validated targets for cancer therapy and diagnostics. Subsequent considerations about possible improvements in the inhibitor's structure re-directed our attention to metallacarboranes as the other type of newly emerging pharmacophores and to carboranes that are substituted by a more tightly binding motif for the zinc atom in the CA IX active site (e.g. sulfonamide instead of sulfamide group). Also other effects (influence of the linker, presence of another group, isomeric effect) on the activity have been studied.

An emphasis was imposed on the progress in the synthesis of cobalt bis(dicarbollide) ion substituted by sulfamide, sulfonamide or sulfamoyl groups. During this research, the scope of methods for flexible C and B- site-directed modifications on metalla(carborane) has been substantially extended by design of building blocks with terminal alkylamine, methylsulfonic, rhodano, isothioureia and other groups used further in 2 to 6 step procedures leading to active inhibitors.

Parallel work on the chemistry of carboranes resulted in the development of two distinctly different new synthetic methods to sulfonamide substituted carboranes. The initial series, comprising a sulfonamide group attached by aliphatic linker of variable length to the boron cluster, was prepared by the insertion of acetylenes into the readily available *arachno*-decaborane system giving a series of mono- and disubstituted *ortho*-carboranes. Later, more flexible and direct routes to these and related carboranes have been developed, providing a full family of isomeric carboranes with one or two alkylsulfonamide groups and a wide spectrum of site-directed substitutions on the cluster.

The new generations of polyhedral borane inhibitors of CA-IX described above exhibit significantly enhanced *in vitro* activities with corresponding K_i values in the low nanomolar range to approximately 20 pM; the best Selectivity factor for CAIX/CAII of up to 668 being observed for metallacarboranes and that of 1312 for carboranes with alkylsulfonamido substitution. The structure-activity relationship (SAR) was studied using a library comprising over 70 new polyhedral inhibitors of CA-IX prepared and tested within this period (ca. 85 in total). These results were complemented by approximately 48 synchrotron X-ray structures of enzyme-inhibitor complexes measured and refined at IMG CAS that covered all promising types of inhibitors, and theoretical and physicochemical studies. The structural studies provided a rationale for the compounds' enhanced activities. In addition, pharmacologically relevant factors such as plasma protein binding, cell membrane penetration, toxicology and pharmacokinetic studies (mice model) were performed on a panel of the selected inhibitors in external collaboration with the Institute of Molecular and Translation Medicine at Palacký University, Olomouc, Czech Republic (IMTM). The tests have shown a significant reduction of tumour size shown on two cancer lines when the animals were treated by selected members from both, carborane and metallacarborane families. Due to their promising properties, these compounds are primarily considered as good candidates for drug development applicable in cancer treatment. Nevertheless, due to their high selectivity for tumour-associated transmembrane CA-IX protein, these boron species may also offer a reasonable potential for future use in imaging methods and BNCT treatment.

Summary of key results during evaluation period

- Versatile procedures for modifications of carboranes and cobalt bis(dicarbollides) in various new directions have been developed. Particular attention was paid on design of

building blocks based on C- substituted carboranes and cobalt bis(dicarbolyde) ion, which were then incorporated into the structures of CA-IX inhibitors.

- Synthetic methods for C- and B- site modifications of (metalla)carboranes by sulfamido, sulfonamido and sulfamoyl functional groups have been developed that involve 2-6 reaction steps from commercially available starting boron compounds. Mono and disubstituted compounds were prepared in each family. Several geometric isomers, prepared by different methods, became available for sulfonamide substituted carboranes. The metallacarboranes with two sulfamido and sulfonamido groups on the cage were prepared as two pure diastereoisomers.
- As verified at IIC CAS, the inhibitors are synthetically available in multi-gram quantities by easy to scale-up syntheses; some carboranes were prepared also in ¹⁰B enriched form.
- Asymmetric substitutions of metallacarboranes with one zinc binding motif and another arm with terminal NH₂ or OH group offer background for further design of drug delivery systems, molecular probes and for tumor imaging. Carborane families might offer potential for combined therapy involving also BNCT treatment.
- The collaborating team of co-investigator from IMG AS CR (IMG) developed new experimental methods for *in vitro* enzymatic screening tests on inhibition for CA-II and CA IX isoenzymes. Positive hits from initial screening were reflected in optimization of the structures of inhibitors at IIC CAS. Tests of enzymatic activity have shown highly specific CA IX inhibition compared to CA II (and other isoenzymes) and observable SAR with activity in low nanomolar or even picomolar range. In respect to substitution, the activity increased in the order sulfonamide > sulfamide >> sulfamoyl group. *In vitro* activity of larger in size metallacarboranes is generally better than that of single-cage carboranes with the same substitution.
- 48 structures of enzyme-inhibitor complexes measured and refined by IMG CAS provided clear rationale for improved potency of the each family of inhibitors.
- Theoretical, modelling and physicochemical studies have been performed in parallel to experimental results. Modelling studies helped to refine x-ray structures of enzyme-inhibitor complexes in case that linkers and auxiliary substituents could adopt several conformations similar in energy and showed features important for understanding of noncovalent interactions of substituted cages with biological targets. These studies have been carried out at IMG in collaboration with members of the IIC team (D. Hnyk) and members of the Group of P. Hobza at IOCB (chemical calculations).
- The compounds, which had been tested *in vivo* show low toxicity and favorable pharmacokinetics. In addition, positive outputs from *in vivo* antitumor tests carried out at IMTM have been obtained. These show significant decrease of tumor grow during period of 24/ 32 days of administration of three selected compounds.
- Some attention has been paid on development on systems useful for *in vivo* imaging studies and drug delivery systems.

Prospects. We will further focus on the use of boron cluster compounds for inhibition of CA-IX and CA-XII enzymes, and aim to develop systems suitable as diagnostic tools and double-action compounds suitable for Boron Neutron Capture Therapy (BNCT). We have discovered recently (in close collaboration with IMTM Olomouc) that some boron compounds cross gastrointestinal and/or blood brain barriers and can serve as drug delivery systems for cancerostatics and antibiotics, e.g. Doxorubicin. In last two years, under a running joint grant project, we have synthesized a series of potentially interesting compounds and investigated structural factors that enable compounds to cross the biological membranes/ barriers, diversifying the compounds into several categories and trying to identify putative transporters responsible for active transport.

Another aim is connected with the electrochemical labelling of biomolecules. Results published over the evaluation period and in early 2020 showed that substituted cluster compounds can be used for design of electrochemical labels for biologically active compounds and biopolymers. Currently we collaborate with the Biophysical Institute CAS CR in Brno on design of reactive molecules and new types of reactions, particularly 3+2 cycloadditions that would allow efficient electrolabelling of large biomolecules.

2. Luminescent boranes

We have recently become interested in the photophysical properties of the binary borane *anti*-B₁₈H₂₂, the centrosymmetric isomer of octadecaborane(22). Of all the known binary boranes (compounds consisting only of boron and hydrogen), *anti*-B₁₈H₂₂ is the only one to have the property of inherent fluorescence. Previous to this evaluation period, in 2012, we described a full and comprehensive examination of the photophysics of the borane *anti*-B₁₈H₂₂, revealing it to exhibit an intense blue fluorescence with a quantum yield approaching unity ($\Phi_F = 0.97$). In the beginning of the current evaluation period, in 2015, we discovered that solutions of *anti*-B₁₈H₂₂ show laser emission with a good efficiency and an outstanding resistance to photodegradation when compared to commercial organic laser dyes; making it the first laser borane. Our current research is expanding on these promising beginnings, seeking to fully understand the potential and limitations of this and other new luminescent borane molecules, and novel borane nanoparticles, at a fundamental level with an aim to maximise laser efficiency and photostability, and offer emission at tuneable wavelengths. We are achieving these goals by forging a comprehensive understanding of the photophysics and photochemistry of *anti*-B₁₈H₂₂, and investigating the effect chemical substitutions and derivatizations have on the absorption and emission characteristics of this and related boranes. During this evaluation period we have made significant progress in revealing the excited-state chemistry of *anti*-B₁₈H₂₂ and demonstrated that its photophysics may be modified by the introduction of substituents or ligands to replace the terminal hydrogen atoms on the cluster. Examples are -SH, methyl, iodine, and pyridine which give molecules capable of, for example, environment-sensitive thermochromic luminescence, single-molecule multiple emissions, polyhedral swelling, and the photosensitization of oxygen.

Ultimately, the successful completion of these aims will further the prospects of the boranes as a novel and competitive alternative to present organic laser dyes and luminescent materials.

Our team are the source of all the ideas in this area, we are the main corresponding authors of the work published, and we perform all the synthetic and structural characterisation work as well as the majority of the photophysical studies. Centres in Praha, Bratislava, and Spain (Madrid and Valencia) have provided valuable assistance with advanced/specialist photophysical experimental work (2-photon absorptions, laser optics, etc) and computational studies, making our contribution multi-disciplinary in its nature, linking novel inorganic chemistry with physics.

Why the boranes? Boron has one valence electron fewer than its number of valence orbitals. This makes boron the only electron-deficient non-metal, and necessitates hydrides of boron to share electron density in spherically-aromatic delocalized fashions. It does this by forming molecules of polyhedral cluster geometries. These geometries represent an architectural "bridge" in the structural continuum that stretches from the highly condensed clusters adopted by the late transitional metals to the open chains and ring formations found in the hydrocarbons. As chemical and physical behaviour is often a function of structure, then the boron hydrides have, with their unique polyhedral molecular geometries and molecular orbitals, large potential for novel properties.

Summary of key results during evaluation period

- **Discovery of *anti*-B₁₈H₂₂ as the first laser borane.** The new borane laser has a resistance against degradation that is superior to many of the commercially available state-of-the-art blue laser dyes. This means that the number of times the liquid medium has to be replaced is reduced, helping to solve issues with costs, occupational hazards and environmental impact due to the handling of solvents that are often toxic and flammable. In addition, the excellent solubility of *anti*-B₁₈H₂₂ in organic environments enables its incorporation into polystyrene polymer matrices without the loss of its fluorescence properties. The resultant new range of *anti*-B₁₈H₂₂/polymer nanocomposite materials with interesting optical properties have potential for use as coherent light sources in optoelectronic, spectroscopic and sensing devices, and as chromophores in luminescent solar concentrators. We published our discovery in Nature Communications in 2015. The paper has been cited 30 times, and significantly contributed to the exposure of our institute to the general public via reference in several popular science publications, international magazines on optics, and Czech national newspapers.
- **Environment-sensitive emission from pyridine ligand derivative of *anti*-B₁₈H₂₂.** The unusually structured new boron hydride, B₁₈H₂₀(NC₅H₅)₂, has an interesting photophysics that is sensitive to both temperature and physical state: Its luminescence being linked to the flapping-like motion of its pyridine ligand “wings.” In combination with *anti*-B₁₈H₂₂, in polystyrene thin-film membranes, wide-range absorption of light (across more than 300 nm) and energy concentration is achievable, a significant spectral coverage possibly useful for luminescent solar concentrators – devices that collect light energy over a wide range of frequencies and concentrates that energy into a single wavelength emission. This work was published in Advanced Optical Materials in 2017 and in Dalton Transactions in 2018 and has been cited on 21 and 12 occasions respectively.
- **Iodination of *anti*-B₁₈H₂₂ to generate efficient photosensitizers of oxygen.** Monoiodinated and diiodinated derivatives facilitate inter-system crossing of excited singlet to triplet states resulting in a high quantum yield green phosphorescence, an emission that can also be excited via non-linear two-photon absorption. This light energy can activate oxygen to produce its highly reactive form: singlet-oxygen, useful in organic synthesis or in the neutralisation of bacteria, for example. This work was published in Inorganic Chemistry in 2019, and has been cited 2 times so far.

Prospects. Our work over the current evaluation period has opened up a new area in luminescence chemistry, that of the boranes. Our contributions offer new molecules, full characterisations of their structures and properties, and the foundations towards their use in functional devices such as lasers, solar concentrators, and oxygen sensitizers. Our work is published in good journals and gaining in impact, with independent groups in China (Chunhua Tan *et al.*, South China Uni.) and America (Alex Spokojny *et al.*, UCLA) also beginning to investigate the photophysics and photochemistry of *anti*-B₁₈H₂₂ and related compounds. Consequently, we are confident and excited of the prospects for luminescent boranes, and we look forward to leading their development.

3. Systematic synthetic chemistry of boron cages, new types of parent and substituted carboranes and main group heterocycles

During the five-year evaluation period we also focused on basic chemistry on boron cages, particularly to these connected with chemistry of tricarbollides, ten vertex dicarbaboranes and aspects of their substitution reactions. This work has been carried out under two subsequent grant projects supported by the Czech Science Foundation.

Summary of key results during evaluation period:

As to tricarbollides, an unexpected rearrangement of carbon vertexes was observed that was studied also computationally; such a rearrangement proceeds via two transition states and in an usual manner that could have implications in polyhedral systems other than just the boranes/carboranes. The 8-R-*nido*-7,8,9-C₃B₈H₁₁ tricarbollides were also successfully alkylated on the open pentagonal face, the resulting mono- and dimethyl derivatives were structurally characterized using NMR spectroscopy and X-ray diffraction analyses.

Alkylation of other carboranes were also investigated. When reacting *arachno*-6,9-C₂B₈H₁₄ with acyl chlorides ROCl, for example, the product strongly depended on the nature of R. The majority of acyl chlorides gave 8-R-*nido*-7,8,9-C₃B₈H₁₁ species, but bulky acyl chlorides (*adm*, *mes*, *naph*, *anth*) induced unusual cage rearrangements to give derivatives of *closo*-1,6-C₂B₈H₁₀. The explanation of such a stereospecificity that dictates these reaction pathways belongs amongst the greatest achievements of Bohumil Štíbr.

Another dicarborane, *nido*-5,6-C₂B₈H₁₂, in particular its 6-alkylated derivatives, were shown to undergo click dehydrogenation reactions to give 6-R-*closo*-1,6-C₂B₈H₉ with bicapped-square antiprismatic shape. Such a dehydrogenation method can be viewed as a general route to alkylated carbon-vertex derivatives of R-*closo*-C₂B₈H₉ systems, which are potentially useful for the development of the carborane-based pharmaceutical and surface-modification work being pursued at the IIC.

DFT computations of related halogenated carborane systems were used to explain the mechanism of experimentally evident rearrangements the in X-*nido*-5,6-C₂B₈H₁₁ (X = Cl, Br, I). Five transition states were calculated as being necessary to convert 7-X-isomers into 10-X-isomers. It was additionally discovered that in the case of [*nido*-5,6-C₂B₈H₁₁]⁻ a molecular tautomerism is evident that has no precedence in the whole area of carborane chemistry. The skeletal tautomers of 5,6-dimethyl derivatives of this species were isolated and they differ in the positioning of the cluster open-face hydrogen atom bridge, as was confirmed by ¹¹B NMR spectroscopy and X-ray diffraction analyses.

Electrophilic halogenation of *closo*-1,2-C₂B₈H₁₀ revealed that substitution occurs at the B7 and B10 vertices, which are the positions furthest removed from the CH cluster vertices. The course of the halogenation is strongly dependent on the nature of the halogenation agent and reaction conditions. Individual reactions led to the isolation of monosubstituted compounds 10-X-1,2-C₂B₈H₉ (where X = F, I) and 7-X-1,2-C₂B₈H₉ (where X = Cl, I). Disubstituted carboranes 7,10-X₂-1,2-C₂B₈H₈ (where X = Cl, Br, I) were obtained under more forcing conditions and their structures were established by X-ray diffraction analyses. Electrophilic methylation of similar carborane systems led to the isolation of methyl camouflaged dicarboranes *closo*-1,2-, 1,6- and 1,10-H₂C₂B₈Me₈ in high yields. The permethylated compounds exhibit high air stability in comparison to unprotected counterparts as a consequence of their rigid, egg shaped hydrocarbon structures incorporating an inner C₂B₈ carborane scaffolding. The structures of all compounds isolated were confirmed unambiguously by multinuclear (¹¹B, ¹H, ¹³C, and ¹⁹F) NMR measurements, supported by X-ray diffraction analyses and computations of the molecular geometries and ¹¹B NMR chemical shifts. Again, these discoveries in basic carborane synthetic methodology we hope to integrate into our synthetic plans for novel pharmaceutically useful boron-based molecules.

A further endeavour of ours has been the delineation of new aspects of ¹¹B NMR spectroscopy, which is an integral tool in the structural characterisation of borane/carborane clusters, often being the method of choice in boron cluster chemistry. Here, we have shown the principles of a new α -shift correlation (ASC) NMR method that we demonstrated on a model series of substituted derivatives of *o*-carborane for which reliable NMR data were available. This graphical method revealed an acceptable linear correlation between α (¹¹B) or α (¹³C) shifts and those induced by substituents in unsubstituted positions of the carborane cluster. The ASC method appears to be an important tool for quantitative assessment of substituent NMR effects in all *exo*-substituted boron-cluster systems.

Chemistry of main group metal heterocycles. This present newly opened area of chemistry at the IIC, introduced by Oleg Tok, has focused on the synthesis of a new family of heterohelicenes, built from fused siloles, with up to 20-30 condensed heterocycles - the longest helicene known so far. We are expecting to obtain new types of saturated and unsaturated (poly)heterocyclic compounds containing B, Si, Ge, Sn, O, S, and N in various combinations. These compounds are potentially useful for molecular electronic, capacitors and Si-C composites.

Prospects. The main objective of future work will be to continue in our efforts to extend synthetic methodology in basic borane/carborane chemistry. For example, further development of permethylation or persubstitution strategies in the area of 10-vertex dicarborane skeletons as well as other selected carborane and heteroborane systems, thus taking initial steps towards breaking the reactivity barrier of these extremely stable and inert compounds and forging routes to the construction of molecular shapes of planned design. Special attention will be paid to the derivatization of the methyl substituents on the peralkylated carborane cages, as well as cage opening/cage degradation/aufbau, and possibly metal incorporation procedures. The compounds represent a new genre of molecular architecture with great future potential in the fields of medicine, molecular biology, luminescence or liquid crystals technology.

4. 2D Carborane self-assemblies

We focus on investigating carborane molecules and related borane clusters as constituents of 2-dimensional self-assembled monolayers for their several advantageous features: First, they exist in a number of isomeric forms with practically identical geometry but different physical properties such as dipole moment magnitude and orientation. Secondly, they exhibit high chemical and thermal stability, and, thirdly, due to the low conformational freedom of their relatively rigid skeletal structures, they show a lower total number and fewer types of defects in their 2-D assemblies. All these mutually related aspects contribute to our longer-term interest in this molecular system and its use to both better understand selected self-assembly principles as well as to make new types of materials with atomic precision. Our interest in this area started in the 2000s with the first article published in Langmuir in 2005 presenting the fundamental characterizations of carboranethiol-modified surfaces of both gold nanoparticles and flat films, and this has brought us several high-quality international collaborators and projects, as stated later in this report. In the period of 2015-2019 we concluded and published several studies which shed more light on aspects such as the interaction between these molecules' anchoring thiol groups and a flat gold surface revealing clearly the existence of two binding modes associated with their inherent SH bond scission aptitudes. At the beginning of this evaluation period we also presented the first bi-functional derivative, *para*-carborane cluster analogue of *para*-mercaptobenzoic acid, which possesses higher symmetry compared to its "sheet-like" organic counterpart. This highly symmetrical molecule was shown to densely pack on surfaces in a hexagonal pattern and the nearest neighbour distance of ~ 7.2 Å. In addition to forming nearly defect-free self-assembled monolayers (SAMs) we have succeeded in opening up carborane SAMs to further chemical interactions. However, to better understand all our contributions to this area it is important to mention the complexity within which all the particular evaluated articles and results are to be understood. SAMs, their structure, stabilities and properties are the result of a number of interactions: the interaction of the molecules with the surface; molecular stability which is influenced also by its exo-substituents; lateral, short-contact intermolecular interactions; lateral, non-binding interactions such as long-distance reaching dipole-dipole forces; and also interactions in the longitudinal direction which can modify the electronic structure of the SAM. We have achieved a very good level of understanding of some of these basic aspects, and some of our experimental discoveries and observations, despite being on the front-edge of knowledge, represent with their convincing clarity almost textbook examples. To further develop on the laid foundations and advantages of these unique molecules we have focused

on investigating dipole-dipole interactions within self-assembled materials and Tomáš Baše was awarded prestigious Fulbright-Masaryk support for his stay at UCLA in 2019.

As part of our general scientific effort we have put together international teams of experimentalists and computational scientists covering all the necessary fields ranging from syntheses and characterisations of new building blocks to surface investigations by surface-sensitive techniques, and we successfully use this synthetic (man-made) molecular system not only to make new materials but to better understand mechanisms of self-assembly and achieve a comprehensive understanding of this complex area.

The role of the Czech boron team (Department of Syntheses) in developing this field: Our team is the key player in this field, we initiated the use of carborane molecules as SAM components, and have significantly contributed to putting international groups together with the common goal of pushing the boundaries of this field forward. We are one of the corresponding authors on most of the essential studies published in this area, we perform either most or all of the synthetic and structural characterisation work, we have also done some of the surface experimental (XPS) and computational characterisations and we are responsible for the results rationalizations and writing up the results. With our international collaborators we aim at combining our skills, methods and scientific approaches in a complementary way employing different techniques (ambient or UHV STM, surface diffraction techniques, complementary computational methods) to achieve a comprehensive picture of the assembled molecular materials.

Summary of key results during evaluation period

- **Molecules do the can-can.** Through investigation of two isomeric species of carboranes we have achieved very good understanding of how carborane-dithiols interact *via* their SH groups with a gold surface. There is a simple acid-base chemistry to control the way these two isomeric species are adsorbed on a surface. The artistic expression of molecules doing the can-can comes from how they “stand on” either one or both anchoring groups, the other one being lifted up (i.e. physi-sorbed). The differences between both isomers, as observed by STM on a surface, correlated very well with the measured qualities of both species in their bulk form (pK_a values, SH NMR shifts, computational results).
- **COOH terminated carborane SAMs.** The first preparation was made of the *p*-carborane cluster analogue of *p*-mercaptobenzoic acid as a building block with pentagonal symmetry for metal surfaces. We show the investigation of pristine as well as mixed (carboxylated and non-carboxylated) carborane monolayers. The effect of the COOH group as an additional substituent on the adsorption and SAM array was analysed. A fraction of the molecules was found to be adsorbed as thiols and proved labile to thermal desorption. This study is the first which introduces a bi-functional carborane molecule and studies the additional substituent effect on the sorption behaviour. This work was published in Chemistry of Materials in 2015 and highlighted on the journal’s front cover. Over the evaluated period we have also prepared and fully characterized two isomeric species analogues to meta-mercaptobenzoic acid with greater lateral steric demands and further focused on the characterization of COOH groups embedded in the SAMs and their reactivity. One student has completed his bachelor and master theses, focusing on the synthesis, characterization and XPS investigation of modified surfaces. Submission of these related results fell into 2020.
- **Building blocks for other than 2-dimensional self-assembled materials.** This study analysed and compared the steric demands of 12-vertex and 10-vertex di-carboxylated carborane ligands as MOF linkers similar to the prototypical terephthalic acid. Highly porous and robust crystalline PCP species of zinc (rob net) and cobalt (‘quasi’ pcu) as well as a topologically unexpected copper compound (lvt) defined the individuality of the 10-vertex carborane cage as a new fundamental spacer type for MOFs. One interesting result is represented by finding a new and unusual Cu-MOF topology while addressing

the fundamental question of the difference between the linear, di-carboxylic 12-vertex and 10-vertex carborane ligands.

Prospects. Carborane and other boron-based cage molecules represent a unique chapter of chemistry. In addition to our passion to better understand the basic aspects of these molecules we aim to develop the advantages they offer towards application in fields of much broader interest such as self-assembled materials. Over the evaluated period of 2015-2019 we have made progress in the accurate mapping of dipole-driven patterns within SAMs while keeping the basic geometric pattern identical or nearly identical. Our contributions provide insights into the principles of self-assembly as well as generate new materials of increased stability and unprecedented adjustability. We will further build upon these foundations and assume growing interest in these molecules going hand-in-hand with their better availability, and we will continue publishing our findings and discoveries in excellent science magazines by the main science publishing houses (ACS, Nature, Wiley, Elsevier).

5. Computational chemistry focused on polyhedral boron compounds

Following from the previous evaluation period, worked continued on the σ -hole studies of the heteroborane 12-ph-*closo*-1-SB₁₁H₁₀ and its various reactions with carbenes and bases. A series of new thiaboranes of *nido* electron count with negative heats of formation was computationally suggested beyond the icosahedral barrier. Additional attempts to find for further types of σ -hole interactions that are responsible for crystal packing forces in heteroboranes were successfully made. A novel stibacarbaborane, i.e. *nido*-7,8,9,11-Sb₂C₂B₇H₉ was prepared and in light of the computation of the electrostatic potential (ESP) a unique σ -hole was found in the middle of the Sb-Sb vector, characterized by the currently most positive value of ESP, 42.7 kcal.mol⁻¹ in the large body of heteroboranes, and a very strong pnictogen bonding in the corresponding crystal was thus revealed. In contrast, in a series of brominated carboranes, the icosahedral clusters' strong halogen bonds in their crystal structures were computed and shown to be due to bromine substitution of the hydrogens bonded to carbons. Dihydrogen bonding played its role in the crystal packings as well. Icosahedral structural motifs both with inner substitution of BH vertices with phosphorus atoms and with *exo* substitutions of terminal hydrogens with chalcogens (Se, Te) revealed very strong pnictogen and chalcogen bonding, respectively towards 2D aromatics in the corresponding co-crystals with them. Some of the *exo*-chalcogenated icosahedral carbaboranes were successfully electron-diffracted, and in conjunction with *ab initio* and DFT computational protocols their gas-phase structures were derived. These experiments were carried out at the University of Bielefeld, Germany. 9,12-I₂-1,2-C₂B₁₀H₁₀ was also successfully diffracted there and diffraction patterns were computationally analysed as a consequence.

We have also reinterpreted the nature of B-H... π contact between the icosahedral carborane and phenyl in metal-containing complexes. We unambiguously confirmed that this kind of non-bonded interaction is exclusively due to attractive dispersion energy term and not a new type of electrostatically driven nonclassical hydrogen bonding.

The nature of bonding in a series of heteroboranes was examined by means of newly introduced approach, namely Intrinsic Bond Orbitals (IBO). Such analyses revealed, among known 2c2e and 3c2e bonds, also 4c2e bond. These electronic features were related to experimental data, dipole moments are among them. All these computational efforts were due to a very productive liaison with the group of Pavel Hobza of the Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences.

The multidimensional statistical method known as factor analysis was applied to a series of BX₃ compounds that are experimentally available. Their experimental ¹¹B NMR spectra agree well with the computed data and this statistical approach provided a deeper insight into the nature of the magnetic shieldings in this series, the magnitude of the ESP (related to π -

hole magnitude) on the central boron atom being one of them. Occupancies in 2p orbitals as well as energetic criteria and Taft inductive parameters are the others. π -holes on boron atoms presented in BI_3 dictates its trigonal planar crystal structure as opposed to trigonal pyramidal AsI_3 , in which σ -holes on As are presented.

1,2-dicarboxy-*o*-carborane was computationally predicted to act as a superacid both in the gas and liquid phase, the corresponding acidities being far beyond those related to H_2SO_4 .

The weakly-coordinating anion, *closo*-1- $\text{CB}_{11}\text{H}_{12}^-$, stabilized π -complex of the hydronium ion with toluene as revealed by the second derivative analysis performed on the basis of the crystal consisted of hydronium, toluene and *closo*-1- $\text{CB}_{11}\text{H}_{12}^-$.

The transmission of electronic effects through *p*-carborane was also computationally examined and it was concluded that this cage acts as a slight π -electron acceptor.

The experimental observations in terms of complexing a macropolyhedral $\text{B}_{21}\text{H}_{18}^-$ (two joint icosahedra via three naked boron vertices) with cyclodextrines both in the gas and liquid phase (water) were successfully accounted for computationally, in conjunction with the Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences.

Finally, computational work was used to help explain the various phenomena when a boron cluster interacts with an electromagnetic field were undertaken. The $\text{Li}@\text{B}_{10}\text{H}_{14}$ complex was reinvestigated and it was concluded that it has a moderate nonlinear optics response in contrast to the original statement. Its response to a magnetic field was also reinvestigated with the correct description of the ^{11}B NMR pattern of an open shell system. 30 isomers of so far unknown $\text{B}_{26}\text{H}_{30}$ were structurally derived and the most three stable ones were found to be very promising candidates as boron lasers and dopants in semiconductor industry. The *trans*- $\text{B}_{20}\text{H}_{18}^{2-}$ and *photo*- $\text{B}_{20}\text{H}_{18}^{2-}$ system was characterized as the first boron photochrom since the experimentally known photo/thermal rearrangement was tackled computationally with the relevant conclusions.

Prospects. All the above mentioned efforts will continue, with an emphasis on materials science that might follow from noncovalent interactions in heteroborane crystals. In effect, due to the 3D spherical shape of heteroboranes, they are suitable candidates to create crystal structures that possess cavities, which might be used, for example, for adsorption or separation. This makes them analogous to e.g. zeolites, but in contrast to zeolites, the network is formed by molecular entities with low molecular weight that are connected by noncovalent interactions. Preliminary results have shown that the size of a cavity in the crystal structure of 12-phenyl-*closo*- $\text{SB}_{11}\text{H}_{10}$ can be substantially increased e.g. by introducing the $\text{N}(\text{CH}_3)_2$ group on the phenyl ring. The synergy effect of the interaction of partially negative NMe_2 and B-H groups with the positively charged part of the quadrupolar molecule such as CO_2 could lead to selective adsorption of this environmentally important pollutant. The investigation of the host-guest systems as well as insertion of heteroboranes into cavities created by biomolecules might have consequences in medicinal chemistry. Modern computational protocols will be extremely useful in terms of assessing the possible applications of these unique class of materials.

6. Industry targeted research

We have continued in collaboration with an industrial partner Katchem, s.r.o. in the Czech Republic on developing original methods for the production of borate ions and their derivatives within the framework of a project funded by the Technology Agency of Czech Republic (TA ČR). Salts of the $[\text{B}_{10}\text{H}_{10}]^{2-}$ and $[\text{B}_{12}\text{H}_{12}]^{2-}$ ions and their derivatives are widely applied as solid electrolytes for batteries, in drug design, boron neutron capture therapy, catalysis, and in the automotive industry. We have developed new methods for the production of $[\text{B}_{10}\text{H}_{10}]^{2-}$ and anhydrous $\text{M}_2\text{B}_{12}\text{H}_{12}$ or $\text{MB}_{12}\text{H}_{12}$ ($\text{M} = \text{Li}^+, \text{Na}^+, \text{Mg}^{2+}, \text{Ca}^{2+}$). The IP generated was protected by CZ Patent Application. We assume, this know-how will be

subject of larger scale verifications and licensing. Licensing of the new technology would also enable continuous supply of basic boron chemicals, similarly as in previous years.

Another industrially directed project was focused on development of carboranes with convenient anchoring groups for their immobilization on silicon surfaces. The potential use of this system is in advanced electronic devices based on Si semiconductors and modification of surface properties at semiconductor boundaries. Project was supported by the Technology Agency of the Czech Republic (TACR), Program Epsilon, TH01020844. Collaborating partners: Katchem, s.r.o., Institute of Physics of the CAS.

A further project involving thiolated carboranes, entitled "Anticorrosive treatment of silver surfaces with self-organized monomolecular layers", has focused on the molecular protection of silver-coated fabrics using self-assembled molecular layers of compounds developed at the IIC. The Project was supported by TACR, Program COMNID: Support for the transfer of results of applied research into new technologies and services, partial project of commercialization activities DPK-2017-10.

Research activity and characterisation of the main scientific results

Head of the Department: **Kamil Lang**, Research Professor
Deputy Head: **Jiří Henych**, Ph. D.

The Department of Materials Chemistry is composed of 7 scientists (Full Time Equivalent, FTE = 6.5), 1 senior scientist (FTE = 0.5), 4 postdoctoral fellows (one was awarded with the JSPS Postdoctoral Fellowship and is on leave in the National Institute of Material Science, Tsukuba, Japan for two years), 5 PhD students, 1 research assistant, 1 technician, and varying number of university students (December 2019). The team has changed during the evaluated period as one scientist was promoted and at present serves as a leader of the Centre of Instrumental Techniques and one research professor passed away. At present, the team is a stable body comprising several key scientists with clearly defined responsibilities and research plans to pursue good and sound science. The scientists cooperate, share instrumentation and skills within the Department, and publish shared papers. A number of students are working in the Department on their bachelor or master theses within the framework of ongoing cooperation with universities. The IIC is also a training center for doctoral students within joint accreditations with universities, thus 10 PhD students defended their theses on departmental topics during the evaluated period of 2015 - 2019.

Our projects are mostly financed by government-funded programs (2 339 kEUR). It includes the Czech Science Foundation, Ministry of Education, Youth and Sports, Ministry of Industry and Trade (MIT), and Technology Agency of the Czech Republic. Using international funding, we were involved in the European Commission FP project for the development of a device for large scale fog decontamination and the NATO project on nanocomposites for enhanced decontamination of toxic chemicals. Some of the MIT projects were co-financed by industrial partners (Rokospol a.s., Toseda s.r.o.). In addition, a part of applied research was funded directly by our industrial partners (e.g., Škoda JS a.s., NHS s.r.o., Prago-Anorg s.r.o.) (160 kEUR).

Key scientists

Jiří Henych – a scientist who professionally grew up under supervision of research professor *Václav Štengl*, the supervisor of his PhD thesis (VŠ was recognized authority in the field of photocatalytic and catalytic materials, he passed away in December 2018). JH defended his PhD thesis in 2015. In 2017 he won a one-year postdoctoral stipendium from the Program of Excellence for starting researchers awarded by the Czech Academy of Sciences. His postdoctoral stay was at Uppsala University - Angström lab, Solid State Physics Department, Sweden. He was also honoured by the Otto Wichterle Award given to exceptionally outstanding, promising young scientists at the Czech Academy of Sciences. He specializes in the development of *(nano)materials for environmental applications*.

Kaplan Kirakci and Kamil Lang – KK is a French scientist who defended his PhD thesis at the University of Rennes 1 (Rennes, France) in 2006. He is employed at the IIC since 2009 after finishing a postdoctoral stay at University Jaime 1 (Castellon de la Plana, Spain). KL is a research professor of inorganic chemistry. Currently, he serves as the head of the Department and the director of the IIC. Both scientists focus on the synthesis and characterization of new molecules, nanostructured materials, luminescent (nano)materials, and photosensitizers/radiosensitizers of singlet oxygen. The main target is the development of *(nano)materials for photodynamic and theranostic applications, mainly antibacterial and antiviral surfaces, photodynamic therapy, and oxygen sensing*.

Jan Demel – he started his carrier in the IIC as a postdoc of Kamil Lang. Currently, his research is predominantly focused on the design and construction of *coordination polymers, mainly metal-organic frameworks* (MOFs) with permanent porosity. JD developed a new family of ICR MOFs (ICR stands for Inorganic Chemistry Rez) based on phosphinate linkers. Tunable functionalization of these materials leads to increased aqueous stability and

biocompatibility. The materials are investigated for *gas separation and sorption of environmental pollutants*.

Zbyněk Černý – an expert responsible for communication with our industrial partners and all aspects of the intellectual property rights of the IIC. He pursues the development of *construction materials and materials for the nuclear industry*.

Research topics of the Department

Research goals are focused on the design, preparation, and characterization of molecules and (nano)materials with unique properties that can be utilized in industrial, environmental, and medical applications. We seek to delineate the properties of newly prepared materials in detail using a wide range of modern and advanced techniques. University students are actively participating in the research. Some results of our scientific work have also found application.

1. Light-responsive inorganic molecules and materials

We focus on the synthesis and characterization of new molecules, nanostructured materials, and luminescent (nano)materials and photosensitizers of singlet oxygen. The main emphasis is put on their stability, photostability, low toxicity, phototoxicity, and biocompatibility. Our main targets are sensitizers for photodynamic therapy (PDT), radiosensitizers for X-ray induced photodynamic therapy (X-PDT), and photoantimicrobials.

1.1. Transition metal cluster complexes. In 2015, following our discovery that octahedral molybdenum cluster complexes (Mo_6) are potent singlet oxygen ($\text{O}_2(^1\text{O}_g)$) photosensitizers, we opened a new area comprising transition metal cluster complexes with relevant photophysical, photochemical, and biological properties for PDT.¹⁻⁵ In this context, we also investigated Cu(I) cubane clusters and, in collaboration with the Russian (Nikolaev Institute of Inorganic Chemistry in Novosibirsk and Novosibirsk State University) and French (Université de Rennes 1 and Institut Lavoisier de Versailles, CNRS) teams, also octahedral tungsten and rhenium clusters with the $\{\text{Re}_6\text{Q}_8\}^{2+}$ cluster core (Q=S, Se or Te). In most cases, the work was conceived, experimentally performed (syntheses, characterization of compounds and nanoparticles, photophysical and $\text{O}_2(^1\text{O}_g)$ measurements) and written-up for publication by the team of the IIC (K. Kirakci, K. Lang). Our most important national cooperation is with a team from the University of Chemistry and Technology Prague, with whom we have investigated biological effects of our Mo_6 complexes (uptake, toxicity, phototoxicity, etc.). In projects with Russian and French teams we were mostly responsible for photophysical and photochemical analyses as well as being contributors to the writing of manuscripts.

We were the first to apply water-soluble cluster complexes, namely photosensitizers $[\text{Mo}_6\text{I}_8(\text{N}_3)_6]^{2-}$ and $[\text{Mo}_6\text{I}_8(\text{NCS})_6]^{2-}$, to cancer cells under blue-light irradiation.¹ In parallel, we discovered that such complexes can be relevant scintillators and radiosensitizers of $\text{O}_2(^1\text{O}_g)$ upon X-ray irradiation.^{2,3} By combining these properties led us to report the first use of such complexes as radiosensitizers for X-PDT.³ Indeed, nanoparticles made of $(\text{Bu}_4\text{N})_2[\text{Mo}_6\text{I}_8(\text{OCOCF}_3)_6]$ were shown to decrease the dose of X-ray needed to kill tumor cells.³ As opposed to conventional PDT, where these clusters have limited relevancy due to unfavorable visible-light absorption, Mo_6 compounds have high potential in X-PDT due to (a) the practically unlimited penetrability for X rays through tissue, (b) the high atomic number of elements forming the cluster core which increases the cross section of the photoelectric effect, (c) the high quantum yields of the $\text{O}_2(^1\text{O}_g)$ formation, and (d) the versatility given by the choice of apical ligands. We also investigated the phototoxicity of these nanoparticles under blue-light irradiation and reported their unprecedented phototoxic effect with IC_{50} of 160 nM on HeLa cells.

We also reported a strong phototoxic effect for another Mo_6 complex bearing mitochondria targeting ligands with an IC_{50} of 100 nM on HeLa cells.⁴ This complex also photoinactivated

Gram+ bacteria upon blue-light irradiation. As high stability of photophysical and biological properties is necessary for the application of photosensitizers used in PDT and X-PDT, we investigated ways to avoid the detrimental hydrolysis of our complexes that is characterized by the replacement of the complexes' apical ligands by hydroxyl or water moieties. This work led to our development of $[\text{Mo}_6\text{I}_8(\text{OPOR}_2)_6]^{2-}$ complexes, bearing arylphosphinate ligands that display unprecedented water stability.⁵ From this series, the diphenylphosphinate derivate showed an unmatched quantum yield of luminescence (0.67) and $\text{O}_2(^1\Delta_g)$ production (0.93) in aqueous environments as well as a strong phototoxic effect on HeLa cells (IC_{50} 0.5 μM) and no dark toxicity.

To summarize, our work has revealed that: (i) The inorganic core of Mo_6 complexes is responsible for broad absorption and red luminescence bands, long-lived triplet states and high luminescence quantum yields. (ii) The triplet states formed upon excitation with UV, blue light, or X-rays are efficiently quenched by oxygen leading to the formation of $\text{O}_2(^1\Delta_g)$ in high quantum yields. (iii) The deliberate selection of the apical ligands allows for an optimization of the solubility and chemical stability of the metal complexes, especially in aqueous media, which is crucial for biological applications. (iv) We have developed strategies towards the stabilization and optimization of Mo_6 -based complexes for photodynamic applications. (v) We have documented that some Mo_6 complexes are applicable in PDT and X-PDT. Experiments *in vivo* are under way. (vi) Our work has initiated the investigation of other cluster complexes (W_6 , Re_6) in biological applications.

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1.2. Hybrid materials and organized assemblies for photodynamic applications. We have investigated the physico-chemical, photophysical, photochemical, and photobiological properties of new porphyrinoids and porphyrin-based (nano)materials,¹ e.g., covalent organic frameworks (COFs) or conjugated microporous polymers (CMPs),² metal-organic frameworks (MOFs),³ nanoparticles,⁴ and polymeric nanoparticles and nanofibers⁵ where porphyrin units serve as building blocks. The construction enables control of the surroundings of the porphyrin units and prevents their close contacts (aggregation), i.e., provides structural control that, in turn, enables the tailoring for light-induced applications such as photodynamic antimicrobial coatings, PDT, and sensing. The photosensitized inactivation of pathogens has especially become an attractive strategy to combat drug-resistant microbes or viruses. Its advantages encompass a broad spectrum of action, limited damage to the host tissue, and high efficacy, i.e., $\text{O}_2(^1\Delta_g)$ attacks several critical components of bacteria and viruses, thus making their resistance to $\text{O}_2(^1\Delta_g)$ unlikely.

In many cases, the work was conceived, experimentally performed (syntheses, characterization, photophysical measurements) and written-up for publication by our team with the considerable contribution of our two PhD students. Antibacterial activities were analyzed by the team from the University of Chemistry and Technology Prague. We contributed to the work on polymeric nanofibers at the Charles University team with our knowledge of photophysics and synthetic procedures.

We synthesized a number of porphyrinic MOFs and measured their photophysical properties, including the generation of $\text{O}_2(^1\Delta_g)$.³ The tested MOFs had the disadvantage of fast

quenching of $O_2(^1O_g)$ by nearby water molecules or OH groups at the secondary building units. Also, the strong absorption coefficient of porphyrin resulted in only the porphyrin units close to the MOF surface being active. To overcome these limitations, we used several approaches for increasing the $O_2(^1O_g)$ production: (i) Post-synthetic modification at the secondary building unit by a hydrophobic agent³ and (ii) MOF nanoparticles.⁴ We have developed a synthesis of hexagonal PCN-222/MOF-545 nanoparticles, which are proficient in producing reactive oxygen species-mediated apoptosis of cancer cells upon visible light irradiation. The extent of the cytotoxic effect correlated with the nanoparticle size and structural instability. High phototoxicity of the presented nanoparticles and their deactivation within several hours opens the door to possible applications in cancer therapy. (iii) Construction of organic porous polymers – COFs and CMPs, the former materials were the first COFs used for photodynamic inactivation of bacteria and their biofilms.² We showed that porous polymer-based photosensitizers are significantly more potent, in terms of $O_2(^1O_g)$ production, than MOF-based photosensitizers. We redesigned porphyrin-containing porous polymers, so that they better adhere to bacteria.

We developed a top-down process for the fabrication of stable photoactive polystyrene nanoparticles from sulfonated polystyrene electrospun nanofiber membranes with encapsulated porphyrin photosensitizers of $O_2(^1O_g)$.⁵ The nanoparticles have dual function; they are capable of oxygen-sensing and photooxidation reactivity simultaneously. In addition, these photoactive polystyrene nanoparticles have strong antibacterial properties giving them potential as antimicrobial materials or for *in-situ* oxygen-sensing in aqueous media.

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2. New materials for a sustainable environment

This topic focuses on the development and study of materials for environmental applications such as the degradation of pollutants, the sorption of gases, and energy-efficient electronics. These aims encompass the development of green synthetic procedures to conceptually new materials and their composites, some of which are industrially applicable. We also perform detailed studies on the structural, morphological, optical, photochemical, sorption, and catalytic properties of the prepared materials.

The degradation of pollutants is performed preferably under realistic conditions, and combines different approaches to the study of these processes at gas/solid and solution/solid interfaces. Investigated pollutants are mainly organophosphates or phosphate esters, which constitute not only essential biomolecules, but also highly toxic compounds such as pesticides (e.g., parathion, azinphos-methyl, methyl-paraoxon), simulants of chemical warfare agents (CWAs) (e.g., dimethyl methylphosphonate, triethyl phosphate), and real CWAs (sarin, soman, VX, Yperit as an example of non-phosphorus CWA). Due to their toxic risks, the development of fast and effective methods for organophosphate degradation is essential. In the past, several decontamination techniques were developed, however, despite the progress in the field, the degradation efficacy of contemporary materials still does not match the needs in protective applications against organophosphate toxins. Our approach is

based on the development of efficient photocatalytic oxides and their composites and of reactive sorbents with stoichiometric degradation capabilities.

2.1. Nanostructured metal oxides for the degradation of CWAs and environmental pollutants. We have developed and optimized procedures yielding TiO₂/carbon-based photocatalytic composites based on (i) graphene oxide, prepared by the sonochemical approach developed in our laboratory,¹ and (ii) nanodiamonds provided by a cooperating team from the Institute of Physics of the CAS.² The physico-chemical properties of these materials were studied mainly in our laboratory and correlated with their (photo)degradation properties. Operando DRIFTS degradation studies on these materials were conducted by J. Henych in the laboratory of Prof. Österlund at the Uppsala University in Sweden during his postdoctoral stay. The results show that TiO₂-based composites have a strong ability to rapidly adsorb and degrade organophosphate compounds due to their unique surface properties under solar light illumination. We have also delineated how carbon-based components induce structural, chemical, adsorption, and electronic effects that enhance the photocatalytic activity of parent materials.

Nanostructured metal oxides. Besides the most-studied photocatalytic titanium dioxide materials, we have prepared nanostructured metal (hydr)oxides with attractive surface properties using CeO₂, MnO₂, Mg(OH)₂, FeOOH₆ or CuO.^{3,4,5} As an example, Sn-doped CuO nanosheets were synthesized using a solution-phase method, and the influence of tin doping on the morphology and catalytic decomposition of a pollutant model was described in detail. Besides doped single metal oxides and metal oxide/carbon composites, mixed metal oxides and their composites also showed good catalytic activity.^{3,4,5} We have developed water-based procedures to prepare TiO₂/CeO₂ composites, TiO₂/iron oxides,⁵ and mixed oxides/clay minerals,³ where the synergistic effects enhance pollutant decompositions (organophosphorus pesticides, azo dyes, drugs, CWAs and their simulants). We have extended the applicability of these composites to different solvents, dry or humid conditions, and to solution/solid and gas/solid interfaces. We have elucidated the structural parameters that drive the reactivity. We have optimized synthetic procedures for the most promising materials (bare TiO₂, Hf and Zr doped TiO₂, and TiO₂/GO composites developed within the NATO project Green Decon) to assess scale-up from grams to hundred grams with maintaining their efficient decomposition activities.

Carbon-based composites. In the last decade, we have developed and optimized sonochemical syntheses of low-dimensional materials.¹ This includes the preparation of graphene oxide (GO) and its use for the construction of highly active TiO₂/GO composites in degradation of toxic compounds.¹ Furthermore, we developed the sonochemical approach for deaggregation of nanodiamonds (so called salt-assisted ultrasonic deaggregation). Investigation and utilization of this method for obtaining active materials for degradation of target toxic compounds (pesticides, CWAs, etc.) are among our future plans. Nanodiamond-based composites with metal oxides and other materials that we synthesize (e.g., Mo₆ clusters) seem to be perspective ways how to exploit opto-electronic, (photo)catalytic, and sorption properties of nanodiamonds.

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2.2. Porous coordination polymers. Metal-organic frameworks (MOFs) belong to the fast-growing area of organic-inorganic coordination polymers. The structure of MOFs is combining metal nodes and polytopic organic ligands (linkers). The high surface area of MOFs, along with the possibility of introducing functional groups, make them highly promising materials for many applications, including sorption and degradation of pollutants. Even after decades of extensive research, the water stability of MOFs still remains an issue. This lack of information limits the applicability of MOFs in water-based environments.

The work presented here was conceived, experimentally performed (syntheses and characterization) and written-up for publication by the IIC team with the considerable experimental contribution of our PhD students. Structural determinations were performed by experts at the Institute of Physics of the CAS.

Stability of MOFs. One of the drawbacks of many MOFs is their low stability in water that originates from the lability of the coordination bonds towards hydrolysis. For this reason, we focused on (i) a delineation of the stability and degradation mechanisms of Zr-based MOFs (e.g., UiO-66, PCN-222)^{1,2}, and (ii) the preparation of novel MOFs with high hydrothermal stability (see below).³ We concluded that the characterization methods generally used for quantification of MOF stabilities (scanning electron microscopy, powder X-ray diffraction, and adsorption studies) are not conclusive, and that the subtle changes to the structure, indicated by released structural components, have to be analyzed by more sensitive analytical techniques such as high pressure liquid chromatography and inductively coupled plasma mass spectrometry.

We also determined the stability of UiO-66 in water at various pHs. The dissolution of the MOF linker leads to *in situ* formation of new catalytic sites, which correlates with the degradation rate of a model organophosphate pollutant, methyl-paraoxon. We also found that post-synthetic modification of PCN-222 with diphenylphosphinic acid reduces the pore collapse upon water activation, additionally, the hydrophobization of the MOF leads to four-fold enhanced productivity of O₂(¹□_g) under visible light irradiation.⁴

New MOFs. We have developed a new family of MOFs based on phosphinate linkers.³ Phosphinic acids have received only very little attention in the past,⁵ however, since our publishing of the first phosphinate-based MOF in 2018,³ nine additional phosphinate MOF structures have been published to date. We demonstrated that an isorecticular design, well known from carboxylate MOFs, is applicable also for phosphinate MOFs which is in contrast to phosphonate-based MOFs. Thus, the combination of Fe³⁺ ions with the bisphosphinate linker leads to a new MOF, named Fe-ICR-2 (ICR stands for Inorganic Chemistry Rez), with a honeycomb structure. Importantly, Fe-ICR-2 has higher hydrothermal stability than its carboxylate-based analogue Fe-MIL-53. The superior stability of Fe-ICR-2 is related to the stronger coordination bond of phosphinates to hard metals, such as Fe³⁺, in comparison with carboxylate groups. Notably, substitutable groups bound to the phosphorus atom point into the pores of the Fe-ICR-2 structure – thus enabling the fine-tuning of pore hydrophobicity and size via selection of the appropriate phosphinate linker.

Functional MOFs. We investigated MOFs for potential applications in biology and gas storage. We developed novel MOF–polymer composites, with polymers embedded in the pores, for enhanced gas separation. For results on porphyrinic MOFs with photosensitizing properties, please see Section 1.2.

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2.3. Magnetic and thermoelectric materials. Hexagonal ferrites (magnetic iron oxides) have received much attention owing to their applications as permanent magnets, magnetic data storage materials, components in devices operating at radio frequencies, and materials with the magnetoelectric effect. One manifestation of the general trend towards more energy-efficient devices through their miniaturization is in the fabrication of thin films. Therefore, the development of new thin film fabrication techniques for integration of magnetic thin-films remains an important research goal.

Our research activity has included the syntheses of magnetic, thermoelectric, and magnetoelectric materials in the form of oriented thin films and anisotropic ceramics. Mutually enriching cooperation with teams from the Institute of Physics of the CAS and Charles University in Prague enabled us to perform complete structural and physical characterization of newly synthesized epitaxial thin films of hexaferrites.^{1,2} Some of these materials have been studied to date only in the form of single crystals. Thus, our synthetic procedures open the possibility to study these room temperature magnetoelectrics (ME) in the form of thin films. We also synthesized ME hexaferrites in the form of ceramics. Using several complementary spectroscopic techniques, a comprehensive set of information on the spin and lattice dynamics of these hexaferrite compounds in broad frequency and temperature ranges were collected. This information can help in elucidation of the origin of ME in these materials. Thin hexaferrite films also enabled the measurement of the longitudinal spin Seebeck effect within the emerging research of spin caloritronics, which may be regarded as an interconnection of spintronics and thermoelectricity.³

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3. Applied research

Through cooperation with our industrial partners, we strive to transfer the most promising developments in the area environmental applications and original inorganic matrices to commercial ends as summarized below.

3.1 Environmental applications

ZnO-based nanosol for the remediation of insulating facades and plasters, pilot testing. Partners: Malpex, a.s. and Pragothem, a.s.; private funding: 1500 EUR (2017 - 2018). The original procedure for the fabrication of nanosols according to our CZ patent n. 304031 was modified to a waste-free technology. The nanosols are very effective for the remediation of biotically (algae, mold) attacked plasters, facades, ceramics, wood, and concrete blocks. The testing on several public buildings was successfully carried out in 2018 – 2019. We are ready for licensing this technology.

Water stabilization. Partner: NHS, s.r.o.; private funding: 5 000 EUR (2015-2019). We have developed a technology for the fabrication of materials that sanitize and stabilize water based

on natural or artificial substrates with a controlled content of Ag nanoparticles. In 2019, a company submitted a bid to purchase know-how for the technology. It is still under negotiation.

Eco-concretes. Public funding in 2016: 12 000 EUR (Technology Agency of the Czech Republic) The aim of the project was the replacement of Portland cement with new ecological alternatives that reduce CO₂ emissions based on our CZ patent n. 307107. Several pozzolanic and geopolymers-pozzolanic materials on a pilot scale were developed for long-term testing in external conditions. The successful project ended in 2016. In 2019, we started license negotiations with Betonext, s.r.o.

3D printing technology for ecological materials. Original and ecologically sound inorganic systems were designed for 3D printing of materials for robust, high temperature applications. The printed materials are non-flammable, harmless to health, and thermally stable. Our first 3D printed samples were presented at the DENKMAL trade fair in Leipzig (2016, 2018). We tested dosing of the "ink" in cooperation with the Bern University of Applied Sciences, Bern, Switzerland, however, precise and fast dosing of a liquid binder system has not been unraveled so far. Since 2019 we have cooperated with J. E. Purkyně University in Ústí nad Labem on the construction of a printing machine.

3.2. New inorganic materials for higher safety in the nuclear industry. Long-term cooperations with companies are based on our unique know-how in the preparation and optimization of advanced inorganic matrices for nuclear power plant safety.

Core-catcher sacrificial materials. Partners: ÚJV Řež, a.s., Centrum výzkumu Řež, s.r.o.; public funding: 48 000 EUR in 2018-2019 (Technology Agency of the Czech Republic). The sacrificial materials cool, dilute, and remediate molten corium during nuclear accidents. Our materials exhibit extreme stability under ionizing radiation. We also designed new construction materials (e.g., original matrices for low-alkaline concrete needed for safety repository). The project continues in 2020 and 2021.

Inorganic materials for shielding of ionizing radiation. In cooperation with PRAGO-ANORG, a.s. we have developed original non-combustible safety materials for shielding ionizing radiation (ANORGAN). Initial materials were patented (CZ patent n. 304030). Current development is the subject of a commercial secret. The project deals with the preparation of advanced multifunctional ecological composites based on inorganic matrices designed for neutron and gamma radiation shielding with fire protection properties. The products are stable over 1000°C. The products were awarded by the Defence and Security Industry Association of the Czech Republic as the most innovative materials in 2015. The license negotiations for the material production have already started. Private funding: 30 000 EUR in 2014 – 2019.

High-stability concretes. Partner: ŠKODA JS, a.s.; private funding: 120 000 EUR in 2017 - 2019. The precise description of the application work is a commercial secret. We work on the development of special high-stability concretes for nuclear industry (high-strength, shielding and high-thermal conductive systems, behavior and stability under hydrothermal conditions at 150 - 180 °C). The cooperation continues in 2020 (private funding: 6 000 EUR).

Research activity and characterisation of the main scientific results

1. Studies on floodplain architecture

The idea of deciphering the internal structure of floodplain sediment bodies and thus inferring their past development (and floodplain architecture) is not new in places where large outcrops such as clay-, sand-, or gravel pits are present. The actual challenge is working in floodplains without any outcrops, which can be achieved by an integrated approach of sediment coring, geophysical imaging, and geomorphic analysis based on GIS. LEGA has based its work on this topic; on the manual drill-coring and sampling and lab analyses of sediment composition, or in situ analyses in groove cores. Geomorphological (GIS) analyses, geophysical imaging (mainly electrical resistivity tomography, ERT), and OSL dating are performed in collaborations with domestic and foreign experts. **Examples** of research tasks solved in this topic are listed below.

1.1. A bias for the aggradation paradigm has remained perceptible in fluvial deposition studies. The role of human impact (including floodplain aggradation) is commonly overestimated and floodplain sediment reworking is correspondingly underestimated. We have revealed that lateral deposition is more relevant than aggradation even, as in the case of the Litavka River, if the area is impacted by catchment deforestation, ore mining, and failures of settling pond (Faměra et al. 2018; Kotková et al. 2019). Other Czech rivers, which LEGA has studied recently, also show lateral sediment reworking that is more relevant than aggradation (Matys Grygar et al. 2016a, 2016b). The paradigm of human-enhanced sediment accumulation in floodplains triggered by tillage and land cover changes in catchments should be reconsidered and revisited in better planned future case studies.

1.2. Common flaws in fluvial contamination studies have been found that stem from an erroneous understanding of floodplain architecture and the variability in nature of channel sediments. Numerous current methods and routines in fluvial contamination studies are conventional but principally incorrect (critics of major flaws can be found in Matys Grygar 2016, 2020; robust methodologies have been presented in Majerová et al. 2018; Tůmová et al. 2019). We propose that sampling protocols must consider sediment recycling and the high variance in age of sediments, and that sampling at pre-defined distances to channel and depths for sediment sampling without expert-based knowledge should be avoided.

1.3. Fine structure of contamination hotspots. Case studies in the Ploučnice River (Matys Grygar et al. 2016b; Hošek et al. 2018), the Litavka River (Faměra et al. 2018; Kotková et al. 2019), and the Röslau River (Hošek et al. 2020) have showed the usefulness of an integrated approach to floodplain architectural studies. Contaminated floodplains usually have contamination focused in spatially limited hotspots (transversal heterogeneity) and in specific segments along the watercourse (longitudinal heterogeneity). “Textbook” patterns for contamination, such as the decrease of contamination with (i) increasing distance to the river channel transversally, and (ii) increasing distance from contamination source downstream longitudinally, are very coarse. In reality, the contamination is “focused” in sedimentary traps formed by the lateral channel movements of the preceding millennia, centuries, and decades, and by the meander abandonments and formation of levees, as well as the presence of abandoned ponds. In other words, contamination patterns are related to the complex temporal evolution of the floodplain architecture. A limited knowledge on floodplain architecture would lead to an inability to identify the real extent of contamination or its spatial distribution in river valleys. *Vice versa*, a knowledge of the spatial distribution of contamination provides an unprecedentedly detailed image of the actual patterns of fluvial deposition. Historical contamination events, such as radioactive floods with ^{226}Ra and U peaks in the catchment areas of the Ohře River (Matys Grygar et al. 2017a) and the Ploučnice River (Matys Grygar et al., 2016b) or historical mining with a contamination peak in the 16th century, such as Sn mining in the Ohře River catchment area (Matys Grygar et al.,

2017a) produced “cheap” and easily accessible isochronous surfaces in floodplain sediment bodies, whenever possible post-depositional processes are considered. Dating of sediment surfaces is one of the key components in floodplain architectural studies.

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2. Dammed reservoirs and contamination

Reservoirs impact fluvial transport of particles, both as a suspension and as bedload. Each reservoir, however, shows a specific deposition behaviour due to variable bottom topography, water depth, reservoir size, and residential time of water. Typically, the settlement and deposition of particles in reservoirs starts in their inflow, while the finest particles are retained in deeper parts of the reservoir. Fe and Mn oxides are dissolved and re-precipitated in the reservoir; organic matter is retained as well as formed in the water column. All these processes impact the passage of organic contaminants and risk elements, including their artificial portions (contamination) (Majerová et al. 2018; Hošek et al. 2020; Matys Grygar et al. 2020a). The *in situ* chemical reactions (or rather biogeochemical processes) in reservoirs have not been adequately described with respect to their importance to the fate of pollutants in fluvial systems. LEGA, in collaboration with its long-term UJEP and UPOL partners, addressed those issues in the joint project UJEP-UPOL with LEGA being incorporated via external XRF analyses. LEGA have conducted several case studies on reservoirs, which included bottom imaging by sonar, gravity coring, sampling, and organic and inorganic contamination analyses (Matys Grygar et al. 2018a, 2020a). The published study (Matys Grygar et al. 2018a) showed a contamination peak soon after the studied reservoir filling in the 1930s, revealing accumulation of Fe- and Mn oxides in the reservoir basin at higher levels compared to pre-dam conditions, and confirmed considerable retention of risk elements within two reservoirs on the studied river.

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3. Understanding the chemical composition of sediments

Environmental geochemistry studies on sediments must consider grain-size control of their chemical composition, specificity/variability of their provenance, and their possible post-depositional (pedogenic, reductimorphic, or deeper diagenetic) changes. A correct chemical compositional data analysis (CoDA) must respect several fundamental features, which have been hitherto neglected in most preceding and current studies. Bulk concentration of each element c_{raw} is controlled by particle size distribution function (PSD) of sediment bulk through specific PSD and stoichiometry of each individual mineral constituent. Each c_{raw} is impacted by the c_{raw} of any other component, in particular major constituents, through a “dilution effect”, e.g. by variable percentage of organic matter, quartz sand, or autochthonous carbonates. The c_{raw} distributions in compositional data are commonly polymodal and show abundant outliers (their distribution is non-Gaussian), which disqualifies the majority of common statistical tools that use c_{raw} (the least-squares regression, mean, standard deviation, principal components analysis on c_{raw}). The state-of-the-art analytical instruments can produce extraordinarily big compositional datasets, while routine data processing usually devalues them and produces misleading results. **Examples** of research tasks solved in this topic:

3.1. Robust identification of anthropogenic contamination in fluvial sediments requires correct CoDA and takes full account of geochemical-mineralogical principles, as demonstrated in Bábek et al. (2015) and Matys Grygar and Popelka (2016). Incorrect procedures are, however, quite common in published papers (Matys Grygar 2016, 2020). Quantification of contamination (anthropogenic enrichment) and of risk element concentrations needs a full understanding of the natural variability of the sediment composition. The development of novel methodology is essential here.

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3.2. Chemical weathering indices are broadly used in paleoclimate reconstructions based on sediment CoDA. LEGA's major achievements in this subtopic stem from a ten years' study of the Most Basin, Ohře Rift, NW Czech Republic, with several hundred metres of lacustrine sediments taken from ca. 20 drill cores in the last decade. LEGA has shown that the Most Basin hosts the most detailed (continental) archive covering nearly 2 My from around the beginning of the Miocene climatic optimum (around 17.0 Ma); sediments were dated using a state-of-the-art integrated approach of magnetic polarity and cyclostratigraphy (Matys Grygar et al. 2017c, 2017d, 2019a). Due to the absence of fossils and biogenic sediment components, the entirety of useful information from the Most Basin deposits was derived from the chemical composition of its siliciclastic detritic components. Of these, it was mainly the potassium concentrations, recorded for the paleoclimatic signal, that provided the most information (Matys Grygar et al. 2017d, 2019b, 2020b). The raw K concentration had to be corrected for natural variability (as discussed above) as pre-requisites for CoDA, that is achieved by (1) geochemical normalization, (2) joint evaluation of relative K concentrations and proxies for sediment grain-size (Al/Si, Zr/Rb) and clay assemblage composition (cation exchange capacity), and (3) extensive lateral correlation among drill cores covering the basin floor (Matys Grygar et al. 2019b, 2020b). The work on weathering indices in LEGA is continuing and has already brought novel weathering indices (Matys Grygar et al. 2020b) and novel statistical tools in CoDA for paleoclimatic research. The sediment geochemistry logs can be interpreted in terms of climate (or orbital signatures) only if these logs are laterally stable, independent on sediment lithology (Matys Grygar et al. 2020b), and show strong and unequivocal Earth orbital signatures. Neglect of these (apparently simple) requirements can even be found in papers in top-ranked journals (Matys Grygar 2019).

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Research activity and characterisation of the main scientific results

In the evaluated period, the scientific activities of the laboratory can be divided into several sub-areas. The first is the systematic research of clay structures in pigments, which is an original topic with a strong potential for application, and is not studied elsewhere. [1-7] The second area covers other analytical issues related to either (i) individual mineral pigments, (ii) analysis of colour layers in micro-samples, or (iii) non-invasive research of painted artworks, which can be seen as one of the current trends in the research of cultural heritage. [8-12] The last broad topic comprises the experimental study of chemical processes in paints, especially degradation processes, which lead to undesirable aesthetic (e.g., colour fading) and/or technological changes (e.g., loss of layers' cohesion). [13-15] A separate and specialised area, is the transmission of research results towards the interdisciplinary community (restorers/conservators, art historians, monument preservationists) and to the public. These activities included participation in interdisciplinary meetings, organization of interdisciplinary ALMA conferences, and editorial and publishing activities (proceedings and books, also in local - Czech language) [16-18].

1. Clay minerals' microanalysis in paints with application in provenance studies

In our laboratory, more than two thousand micro-samples have been obtained and archived over the 20 years of our activities in cultural heritage research. They were collected from different paintings during their conservation/restoration and represent different historical periods and regional provenances, not only the Central European (Bohemian, Austrian, German, Hungarian), but also Italian, Flemish or Dutch. Such an archive is a key and irreplaceable element in any provenance study that always requires a statistically significant comparison between works of the same period, author or region. Thanks to this advantage, ALMA focuses on provenance studies, which are based on state-of-the-art materials microanalysis combined with an advanced knowledge of historical painting techniques. In general, provenance analysis has to be considered as a complex discipline, combining artistic, historical and materials/technological issues. It includes the authorship and/or workshop attributions, but also period and geographical relationships. For example, natural pigments in historical paintings, which come from various geological sources, seem to be an attractive subject of interest to trace the geographical links. Knowledge of the context, however, shows us that expensive and beautiful pigments, which often attract researchers' attention, have often been imported from remote locations and cannot be used to determine the regional provenance of the artwork. For example, the source of highly desirable blue ultramarine (*lapis lazuli*) for all European painting originated in a single deposit in today's Afghanistan.

For this reason, in our research we have focused on minerals inexpensively available and often used in the preparatory layers of paintings (primers, grounds) that have remained unfashionable – as, e.g., earth (clay-based) pigments. For example, in the Baroque period, coloured clays were largely used for grounds, and since they were cheaply available in many places, it did not make sense to transport them for a longer distance. Also at that time, the painters usually bought the already primed (ready-to use) canvases in the place where they were working. The composition of the clay material may thus be regionally specific - this factor has never been considered by anyone before. In our earliest study in 2015 [1], we first described the distinction between the clay-based grounds of Central European and Italian origins, and differentiated the grounds of the 17th to 18th centuries to 5 distinct types, which most frequently appear. We also verified that anonymous artworks could be classified according to these criteria, thus identifying the region where they were painted. This method very quickly found practical application in the survey of anonymous paintings of the 17th and 18th centuries provided by our ALMA laboratory in museum and castle collections in the Czech Republic and Slovakia. There are numerous copies which were either painted in the place where they are now located or imported - most often from Italy. This method thus

helped to distinguish paintings of local origin from imports, which is very valuable in terms of their art-historical evaluation.

Another reason why this method has not been applied so far is due to missing methodology. The microanalysis of very variable clay minerals' structures, which are the most important part of earth pigments, is very difficult since it should be applied to very small, heterogeneous and scarce fragments from paintings. Because of their rarity, non-destructive analytical approaches are highly preferred. To identify individual clay structures in such a way, it is necessary to apply X-ray powder micro-diffraction (micro-XRPD). We are the only laboratory where laboratory micro-pXRD is systematically developed and tested for the purpose of provenance studies of historical paintings. In 2016, we published a methodology focused directly on clay minerals' XRD analysis in microscale. **[2]** Besides the common limitations of micro-pXRD (such as, e.g., worse resolution, more pronounced effect of heterogeneity in small irradiated volumes, etc.), there are several other phenomena that should be considered in order to avoid a misleading interpretation of micro-diffraction patterns. Most of the special procedures designed to distinguish individual clay minerals (as, e.g., ethylene-glycol solvation, etc.) cannot be performed on micro-samples. Instead, different expandable clay structures can be recognized by characteristic shifts of their diffraction lines, which appear during the preparation of colour mixtures as a result of interaction with organic binders. This discovery was supplemented by model experiments, which showed that proteinaceous binders (animal glue, egg white) cause an increase of the basal spacing (which is relative to the content of smectite layers in the material), while the presence of fatty compounds (in oils or egg yolk) results in its very slight decrease. In both cases, a subsequent dehydration of the structure was prevented. Based on model experiments we are able to reconstruct the original d-spacing of (now modified) clay minerals in colour layers, which is one of crucial factors influencing the correct interpretation of the provenance – and also one of most important findings in this methodological study. Although the laboratory micro-XRPD patterns usually have worse resolution and higher noise than synchrotron radiation X-ray diffraction (SR-XRD), they nevertheless allow calculation from non-basal diffractions - for example, the calculation of crystallinity indices.

We applied micro-XRPD analysis in numerous case studies. We found that, for example, there is a clear difference in the composition of Mediaeval poliments (= preparatory layers for gilding) used in the 14th and 15th centuries and those of the early Renaissance (late 15th and early 16th centuries) showing us that, particularly in various regions of Central Europe (Bohemia, Bavaria, Transylvania, Upper Hungary-Slovakia), alternative raw materials substituted red clays at that time, having a composition more likely corresponding to bauxites than clays. Because this change is quite sharp, it allows some artworks to be more accurately dated. **[3]** Another example of application comes from Slovakia – we found that Master Paul from Levoča, Slovakia, who is considered to be one of the leading artists of late Gothic art in Central Europe, intentionally admixed kaolin to chalk grounds on some of his altars. Based on the presence of alunite (evidenced by micro-pXRD and micro-Raman techniques) the kaolin was specified as hydrothermal, which indicates its possible source in the Tokaj Mts., Hungary. This finding corresponds very well with historical data - at the time of artworks' creation, Slovakia was an integral part of the Kingdom of Hungary and the municipality of Levoča traded with Tokaj wine and also owned vineyards in the Tokaj area, where the hydrothermal kaolin was exploited as well. **[4]**

However, the most important discovery related to the application of clay minerals' microanalysis in paintings is as follows – the composition of the first ever coloured grounds, which appeared in Italy in the turn of 16th and 17th century and which were extensively used by north-Italian painters such as Caravaggio (1571–1610) and his followers, did not correspond to any earth pigment exploited in Italy (despite the fact that there are numerous traditional areas of ochre mining - such as Lessinian Mountains or the Mt Amiata area near Siena), but to calcareous clays. Surprisingly, a material of exactly the same composition was also used by sculptors, which allows us to conclude that the ground material is in fact pottery

clay, and its cheap availability in northern Italy at that time would be related to the increased popularity of terracotta sculptures, which started during the Renaissance, and to the subsequent expansion of pottery clay mining. [5] Speculatively, one can assume that it was the cheap availability that led someone (perhaps Caravaggio himself) to apply this new material for the ground of canvas painting in order to darken the painting and to achieve a strong contrast between light and dark, which is well-known as *chiaroscuro*. It is also a nice example of technological transfer, when the material originally mined for another purpose (ceramics, sculptures) was then subsequently used in another branch (here in painting).

Art historians often believe that the changes in the composition of the materials are solely related to the invention of the painter. Now we can say that this is only partially true. Painters were improving the grounds (additional colouring, re-priming, or, adding a new layer of a different colour). The basic material, however, was a locally available raw material. Matia Pretti (1613–1699) used pottery clays in paintings created in Italy, while weathered reddish *Globigerina* limestones were used instead of clays in his paintings created in Malta. Also the ground of one of the latest paintings by Caravaggio created in Malta (Beheading of St. John the Baptist, Valetta Cathedral) differs from all the previous ones. The dominant components in this case are alunite and hematite, and although the source of this material in the Mediterranean is not yet known, it again represents a regionally specific composition. [6] Of course, other methods have been employed in combination with micro-XRPD, such as the already mentioned micro-Raman spectroscopy, or analysis of minor and trace element via LA-ICP-MS [7]. A very specific area was the development of micro-paleontological procedures in order to determine the geological age of calcareous clays [5].

2. Multi-analytical studies and novel analytical approaches

The conventional approach to the analysis of paint chips (whose dimensions most commonly do not exceed 1 mm) usually includes their embedding in synthetic resin, cutting and polishing to get a cross-section. These micro-sections are considered as “standard” samples as the stratigraphy of the paint layers can be described in them and further analyses can be performed on individual layers. Only in certain cases is the material obtained from the painting large enough to be split thus allowing one of the fragments to be analysed separately without any pre-treatment. Routinely, description of the sample by means of optical microscopy is followed by semi-quantitative elemental analysis (e.g., by scanning electron microscopy coupled with energy dispersive spectrometry – SEM/EDS or micro X-ray fluorescence - micro-XRF), and by spectroscopic analyses of pigments and binders by Raman and infrared microspectroscopy (micro-Raman, micro-FTIR). However, the employment of other methods is still unconventional, such as, e.g., micro-XRPD (already mentioned), or the methods for advanced elemental microanalysis and imaging (PIXE, LIBS, LA-ICP-MS etc.) which occur rather sporadically in the given context. The main limitations are given primarily by the requirement for the non-destructiveness of the analysis or by the limited availability of the material in sufficient purity – it applies also for all the methods of micro-destructive analysis of organic binders (chromatography, mass spectrometry, etc.). In our research, we look for unconventional approaches, which allow us to find highly specific characteristics of the material (with particular interest in inorganic compounds) and thus overcome the routine.

In combination of micro-XRPD and micro-Raman we discovered a way to identify a dark violet variety of fluorite (CaF_2), which is known as antozonite and which was probably the only form of fluorite used in paints. An X-ray diffraction study of antozonite confirmed that the structural disorder caused by natural irradiation may be expressed by the broadening of its diffraction lines. The higher their full-width half-maxima, the lower the sample's lightness. A Raman spectroscopic study showed that antozonites have a specific spectrum that can be used for to distinguish it in microanalysis and in provenance-related studies. As a result, we were able to identify antozonite in Kutná Hora / Kuttenberg Late Gothic altar pieces and to

refine the definition of antozonite, which still remains vague (antozonite = naturally irradiated fluorite). **[8]**

Since the micro-Raman is frequently used for the analysis of synthetic pigments in paints, we decided to test its efficiency for the paintings' authentication according to pigments used. One has to consider, however, that in modern painting it is not a specific pigment that is decisive, but a description of the entire palette of each painter. This is because the availability of pigments is no longer important in modern painting, but the preference in pigment selection and the way of their mixing plays a greater role. This comparative study on mock-ups and famous modern paints by Edvard Munch (1863 – 1944) and František Kupka (1871 -1957) clearly shows that Raman spectroscopy enables the detection of pigments which would remain unidentified (as, e.g., zinc and chrome yellows), but on the other hand, it does not detect all the components in mixtures and its efficiency is surprisingly low. **[9]** Within this study, different laser excitation wavelengths and both the laboratory and portable instrumentations have been compared.

An unconventional combination of analytical approaches (X-ray diffraction - XRD, high temperature XRD, TA/MS, electron microprobe analysis - EMPA, Raman, infrared and UV–VIS–NIR absorption spectroscopies) was also used to identify the colouring agent of natural chrysoprase (Ni-bearing gemstone) from Szklary deposit, Silesia, Poland, which was widely used in the Gothic period by Bohemian art workshops as a decorative stone (Castle Karlštejn, St. Wenceslas Chapel in the Prague's Cathedral). The situation was made even more complex by the fact that some of expected silicate phases (kerolite, pimelite) were just excluded from the mineralogical classification due to the insufficient proof of tightly bound water in their structure. The conclusiveness and quantification of interlayer water was thus one of the key tasks enabling the structure description. This was accomplished by experimental research of reference samples using TA/MS and high temperature XRD. These methods showed that "kerolite" and "pimelite" contain interlayer water, i.e. they differ from the non-hydrated talc–willemsite mineral series. Subsequently, the XRD and EMPA analyses of Szklary chrysoprase indicated that chrysoprase's colouring agent is "pimelite", which was further corroborated by Raman and UV–VIS–NIR spectroscopies. **[10]**

One of the current trends, which is also followed by the ALMA laboratory, is the development and testing of non-invasive analytical methods that allow the analysis of the artwork without the need for sampling. Therefore, we created a mobile laboratory (which includes portable Raman and FTIR spectrometers and hand-held XRF analysers) and focused our research (i) on the specification of the term "non-invasive" (it concerns some methods, in fact micro-destructive, laser-based, such as Raman or laser induced breakdown spectroscopy - LIBS) and on the testing of large-area mapping or imaging methods with the aim to simultaneously collect and interrelate visual and analytical information. Concerning LIBS, several effects occurred as expected, such as the elevated crater rim formation, discoloration, and splinters break-out, which prevent this method to be used on an intact painting surface. These effects were, however, reduced or avoided by using a shortened laser pulse wavelength (UV) and also rather low energy. LIBS remains a method for special tasks where surfaces are damaged or hidden and its micro-destructivity does not matter. It allows deep profiling and has better detection limits for some elements (in the order of ppm) compared to commonly used XRF. It was the only method that evidenced (without any sampling) a small amount of cobalt in the blue Gothic glass mosaic tesserae. **[11]**

Recently, we have been testing a large-area macroscale XRF (MA-XRF) scanning of paintings (newly installed in ALMA), which advantageously complements traditional methods of visualization of internal structure of the artwork (IR reflectography, X-ray radiography). Large-area element maps can be used not only to analyse chemical composition, but also to visualize the underlying layers (underpaints, overpaints, retouches) according to differences in spatial distribution of elements and thanks to a sufficient information depth of the method. In our research, we utilised the advantage of high spatial resolution of the method to study portrait miniatures of the 18th and 19th centuries. These small objects have never been

studied before, because subtle detailed painting in thin layers makes any sampling impossible. Advantageously, together with high resolution imaging, we also adapted some laboratory methods to non-invasive analysis, because these small objects (of the size of a human palm) can be placed in measuring chambers. For example, the procedure was chosen for paintings on ivory, which are very sensitive to any changes of relative humidity. EDS analysis in Environmental Scanning Electron Microscope (ESEM) in the presence of water vapour (instead of nitrogen) was successfully tested. This procedure, for example, made it possible to analyse nitrogen in the painting, which is not possible with other non-invasive methods. It allows the unambiguous identification of Prussian blue - $\text{Fe}_4[\text{Fe}(\text{CN})_6]_3$. [12]

3. Investigation of degradation processes

Degradation processes are another important direction of recent cultural heritage research. The increased interest in understanding the various chemical processes in the colour layer was caused by numerous misinterpretations (iconographic, historical) that did not take into account secondary visual changes in an artwork. The understanding of these chemical processes greatly influences also the way of subsequent conservation/restoration and above all also the conditions of preventive protection of the painting. In our laboratory, we deal mainly with processes causing colour changes of pigments, which were caused mainly by external factors (humidity, salt attack, temperature, light) and then also by blistering and loss of cohesion of paints due to formation of metal soaps, their crystallization, aggregation and migration towards the painting surface. This complex process belongs to most investigated ones, because there is still no clear answer to the question of how to prevent or limit it effectively.

An example of the colour change is the fading (or greying) of the blue vivianite pigment – $\text{Fe}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$, which was observed in many 17th-century Dutch paintings. In Dutch painting, this pigment was used very often in underpainting and painting itself. To explain the process, temperature-related degradation of pure synthetic, as well as partly oxidised, natural vivianite has been studied by HT-XRD and Mössbauer spectroscopy. While temperatures around 70°C are already damaging to vivianite, exposure to 160 °C results in complete amorphisation of both the vivianite and its oxidation products. Furthermore, we proved that temperatures around 70°C can be easily achieved by, e.g., relining of canvas paintings in a traditional way using iron. [13] This result has direct practical implications concerning the modification of restoration/conservation procedures and the precise control of temperature.

On the other hand, research on metal soap formation (saponification) processes is still far from general practical application – despite the fact that saponification occurring in paint layers of artworks represents a serious degradation process that affects both the appearance and stability of paintings. Formation of metal soaps is mostly reported as a consequence of the interaction of lead white or zinc white with oil binder. However, we found distinct symptoms of saponification also in the 15th century paint layers comprising egg yolk binder and lead-tin yellow and experimentally proved that the process is in initial stages even faster with yolk than with oil. [14] We also found that neo-formed soaps are various mixed lead carboxylates containing both palmitate (C16) and stearate (C18) in one crystal structure and the proportion of carboxylate anions is affected by the type of the binder. We found that the C16:C18 ratio in mixed lead carboxylates is higher in egg yolk tempera samples in accordance with the more abundant palmitic acid in egg yolk fat. On the other hand, the lower C16:C18 ratio in emulsion binder correlates with increased content of stearic acid, suggesting contribution of both binders, i.e. egg yolk and oil, to the soap formation. Since the crystal structure of mixed Pb-carboxylates is not sufficiently described anywhere, we decided to synthesise them and create a structural model. Due to numerous difficulties in preparing single crystals of sufficient quality we studied the crystal structure of polycrystalline mixed lead carboxylates of the formula $\text{Pb}(\text{C16})_{2-x}(\text{C18})_x$ ($x = 0; 0.25; 0.5; 0.75; 1; 1.5; 2$), where

C16 and C18 stand for hexadecanoate (palmitate) and octadecanoate (stearate) anions, respectively, by complementary XRPD and ^{13}C and ^{207}Pb solid state NMR (ssNMR). We revealed that both C16 and C18 chains are present in one crystal structure, creating the statistical disorder at the ethyl end of the chains. Based on the ^{207}Pb ssNMR spectra, we revealed two distinct local environments of lead atoms, corresponding to the symmetrically (i.e., (C16)–Pb–(C16) and/or (C18)–Pb–(C18)) and asymmetrically (i.e., (C16)–Pb–(C18)) substituted lead carboxylates, and we confirmed the formation of a holo-directed structure for both the structural motifs. **[15]** The structural models were already applied to identify the neo-formed crystalline mixed carboxylates in a model experiment and we suppose that very soon it will be possible also in samples from actual paintings. The detailed differentiation of metal soaps' structures will allow to better understand the causes of their formation and will be the next stone in the mosaic describing the whole complex process.

4. Interdisciplinary forum

ALMA Laboratory has a long-term strategy how to transmit the results of scientific research into practice. On the one hand, we use new methodological approaches in the practical survey of artworks in cooperation with museums, galleries and private restorers, and on the other hand, we attend and organize interdisciplinary meetings (see below). In this scientific part of the report, however, it is necessary to mention above all the publication outputs, which are either chapters in art-historical proceedings and monographs **[16-17]** or also our editorial activity - publishing of proceedings from interdisciplinary ALMA conferences, where we regularly offer also our own results for practical use. **[18]**

5. Research activities of X-ray diffraction laboratory

The activities of the X-ray powder diffraction laboratory, which is a part of the ALMA laboratory, with regard to external co-operations (out of the main topic of ALMA) could be divided into three groups of co-operating bodies. The first group are co-operations within the Institute. We could emphasize the co-operation with the CIT laboratory on the titanium oxide photo-catalysts that finished with scientific papers. **[19, 20]** The second group of co-operations goes to other Czech academic institutions. One of directions closely related to the ALMA Laboratory topic was the research of building materials, which was performed in collaboration with the Faculty of Civil Engineering of the Czech Technical University in Prague. This collaboration led to several top publications. **[21, 22]** We could mention also the collaboration in the research of ultrasonic preparation of nanomaterials with the Faculty of Science of the Masaryk University in Brno **[23]** or the collaboration with the Faculty of Chemical Technology of UCT in Prague, either on the project of electrochemical synthesis of template nanomaterials based on titanium oxide **[24]** or in the field biomaterials **[25]**. The third group of our external co-operations leads to industrial partners, among them the Institute of Nuclear Research in Řež, Centrum výzkumu Řež (Research center Řež), Preciosa a.s., and Bochemie a.s. play the most important and largest role.

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