JERZY HABER INSTITUTE OF CATALYSIS
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HR EXCELLENCE IN RESEARCH

DETAILED RESEARCH REPORT

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Contents

Statutory Research

Catalytic Materials and Processes for Sustainable Development

1. Experimental and computational studies on selected enzymes of application potential

(Professor Tomasz Borowski, Professor Maciej Szaleniec, Anna Kluza PhD, Mateusz Tataruch PhD Eng., Agnieszka Wojtkiewicz PhD, Justyna Andrys MSc, Maciej Hapke MSc)

For the tH6H enzyme, rapid kinetics studies were conducted using the rapid freeze quench method for the tH6H-Fe(II)-αKG-6OH-HYO complex obtained under anaerobic conditions. Samples frozen at specific time points were analyzed using the Mössbauer spectroscopy method to analyze the course of the reaction. Stopped-flow and rapid freeze quench studies were performed in cooperation with the group of prof. A. Osyczka (WBBiB UJ), while Mössbauer spectroscopy measurements were performed by prof. J. Korecki. In parallel, crystallographic studies were conducted on the tH6H-Ga(III)-SIN-6OH-HYO complex, which is an analogue of the reaction intermediate. A crystal structure was obtained that allows for the analysis of the substrate arrangement relative to the metal in the active center in the mimicry of the intermediate.

In cooperation with the Slovak University of Technology in Bratislava, the stability of S-hydroxyphenylethanol dehydrogenase from A. aromaticum (S-HPED) was characterized as a function of pH and temperature, and the thermal stability profile of ketosteroid dehydrogenase (KSTD1) from R. erythropolis was determined. For S-HPED, based on inactivation data from the range of 25 - 60 ◦C, a one-step thermal inactivation mechanism ("one step-two states") was identified at pH 9.0 and inactivation rate constants were determined under the conditions: i) with added glucose as a stabilizer, ii) without stabilizers. S-HPED activity tests were performed in reactions with 15 substrates. For 10 analyzed compounds, the enzyme activity and its high stereoselectivity were confirmed.

Immobilization of L-DOPA extradiol dioxygenase from Beta vulgaris was performed in nine variants; on silica and cellulose supports functionalized with amino, glycidyl and epoxy groups and without functionalization. Ascorbic acid, glutaraldehyde and divinyl sulfone were used as linkers for immobilization, as well as different pH of activation of functional groups (6-11). Despite reaching 100% degree of protein binding, 4-hour activity tests of immobilizates for each immobilization variant did not show the presence of the product.

Modeling of structures of enzyme-substrate complexes of selected 3-ketosteroid dehydrogenases (KstD) was performed for substrates and inhibitors of the reaction, defining the amino acid environment and determining the energy of enzyme: substrate interaction. Structural differences that may be responsible for the substrate specificity of the tested KstD were determined.

For AlphaFold2 models of two human proteins from the LY6 family, high-throughput molecular docking of a subset of about 5 million compounds from the ZINC20 database was performed. The docking results enabled the selection of a group of several dozen promising compounds to be tested by collaborators from the Uniformed Services University of the Health Sciences, Bethesda, MD, USA.

2. Bacterial biopolymers as source of hydroxyacids for application as HBD components in preparation of deep eutectic solvents (DES)

(Associate professor Maciej Guzik, Ewelina Cichoń PhD Eng., Anna Faruga MSc, Robert Karcz PhD Eng., Joanna Kryściak-Czerwenka PhD, Justyna Prajsnar PhD Eng., Katarzyna Zimowska MSc, Wojciech Snoch PhD)

The project aimed to develop and characterize natural deep eutectic solvents (NADES) as eco-friendly alternatives to conventional, toxic solvents used in industry. The project involved multi-stage research encompassing the synthesis, characterization, and optimization of NADES for applications in biomass extraction processes containing polyhydroxyalkanoates (PHA).

As part of the research, a range of NADES was synthesized using various components, with choline chloride and menthol serving as hydrogen bond acceptors (HBA) and donors such as 1,2-propanediol, urea, sugars, and fatty acids, including those derived from PHA polymers. Special attention was paid to menthol-fatty acid systems, which exhibited thermal stability, low viscosity, and hydrophobic properties. FTIR analysis confirmed the formation of hydrogen bonds between the components, while DSC/TGA studies demonstrated that optimal operating temperatures for most NADES ranged from 100 to 150°C, enabling their use in extraction processes requiring thermal stability.

Mixing tests revealed that NADES based on choline chloride were miscible with water and methanol, classifying them as hydrophilic solvents. In contrast, menthol-based systems exhibited miscibility with hexane and chloroform, making them promising hydrophobic solvents. Polarity tests and Nile red analysis further confirmed that menthol-based NADES are suitable for the extraction of hydrophobic PHA biopolymers. Selected NADES were used in biomass extraction processes rich in PHB and mcl-PHA, achieving high polymer yield and purity while simultaneously reducing process toxicity.

The most promising results were obtained with menthol-fatty acid NADES systems, which enabled efficient PHA isolation from biomass under mild temperature conditions. These NADES exhibited lower viscosity, enhancing mass transfer and extraction efficiency. The study confirmed that the use of NADES eliminates the need for toxic solvents such as chloroform while allowing for easy solvent recovery after the process.

The research results indicate the high potential of NADES as green, sustainable solvents for industrial applications. Their biodegradability, non-toxicity, and the ability to tailor their properties to specific application requirements make them a promising alternative to traditional solvents. Menthol-based systems, due to their thermal stability and hydrophobic nature, open new opportunities in the chemical and biotechnological industries, particularly in biomass extraction and biopolymer production.

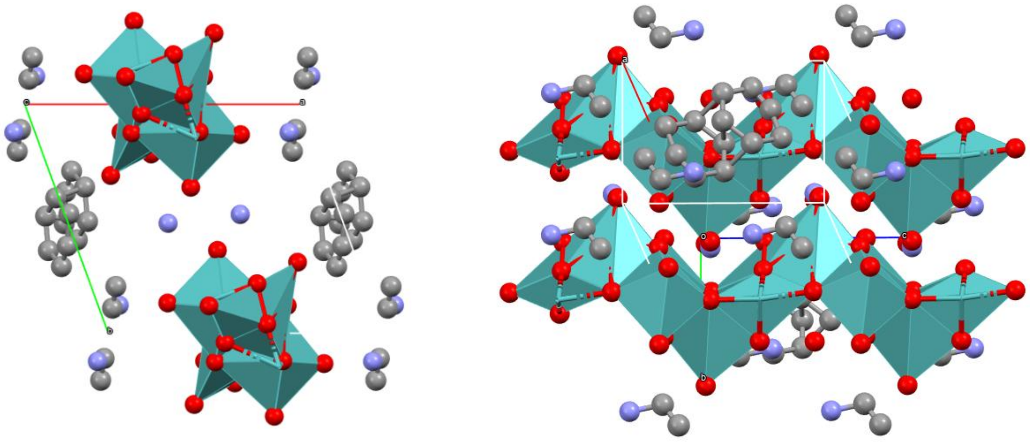
3. New peroxo and polyoxo compounds of Mo(VI), W(VI) and V(V). Synthesis, structural studies and applications in oxidation processes.

(Professor Wiesław Łasocha, Katarzyna Pamin DSc, Daria Napruszewska MSc)

The aim of the research was to synthesize new compounds from the polyoxometalates group, study their physicochemical properties, mainly crystal structures, thermal stability studied by xrpd vs.T methods (powder diffractometry as a function of temperature), and catalytic studies. The tested catalytic reactions were the oxidation reaction of cyclooctane and the Baeyer-Villiger reaction, i.e. oxidation of cyclohexanone with oxygen from air in the presence of aromatic aldehyde. Catalytic studies were supplemented by studies of specific surface area and thermal stability using DSC/TG methods.

Much attention was paid to the research on the preformation of renewable materials, this year these were lignins and the ‘raw’ renewable material, which is grape seeds. The ‘raw’ material was subjected to the process of drying, removal of oil fractions using a Soxhlet apparatus, alkaline baths and final heating in a nitrogen atmosphere. The possibility of saturating the interior of the carbon precursor with a POM’s type material was investigated. This year it was 3,5-dimethylaniline trimolybdate, selected due to the ease and reliability of synthesis in a wide range of conditions (concentrations, reaction time, solution pH). Surface tests, microscopic studies, elemental analyses and attempts to detect POM’s fractions in the obtained materials are proceeding successfully, will be optimized and continued.

Many preparative studies have been carried out to reproduce the synthesis of o-phenylenediamine γ-octamolybdate, copper trimolybdate monohydrate, and to optimize the methods of aniline trimolybdate synthesis. The achievement of a new aniline ammonium trimolybdate is noteworthy. The figure below shows projections of the structure obtained by XRPD methods, not yet refined.



4. Boron nitride as a catalyst and an active phase support

(Associate professor Dorota Rutkowska-Żbik, Professor Małgorzata Witko, Associate Professor Renata Tokarz-Sobieraj, Erwin Lalik PhD, Jerzy Podobiński MSc, Anna Micek-Ilnicka DSc, Małgorzata Zimowska DSc, Katarzyna Samson PhD Eng., Małgorzata Ruggiero-Mikołajczyk PhD, Agnieszka Drzewiecka-Matuszek PhD, Robert Kosydar PhD, Mariusz Gackowski PhD, Łukasz Kuterasiński PhD)

In 2024, a new research topic was undertaken, the aim of which is to explore the reactivity of boron nitride (BN) as a catalyst and an active phase carrier in selective oxidation reactions. Our interests were driven by the growing interest in BN in catalysis, which has been observed in recent years.

The boron nitride powder produced by Acros Organics was selected for the study. Next, the sample was activated in plasma to modify its surface properties (BN_{plasma}). Both preparations were characterized by basic physicochemical techniques (XRD, SEM, low-temperature nitrogen sorption, ^{11}B and ^{1}H MAS NMR).

It was found that the specific surface area of the initial BN decreases as a result of plasma activation (from 42 m^2/g to 36 m^2/g). The pore distribution determined using the NL-DFT method also changes: while in BN the pores with a diameter of 284 Å dominate (the others are 179 Å and 199 Å), after modification in plasma the pores with a diameter of 199 Å dominate.

The samples prepared in this way were used as catalysts in the oxidative dehydrogenation of propane (ODP) and carriers of the active phase: heteropolyacids (HPA).

ODP tests were performed in a fixed-bed gas flow reactor connected to a gas chromatograph to detect substrates and products, using 0.5 mL of catalyst mixed with quartz in a 1:1 ratio, at a reaction mixture flow rate of 60 mL/min. In the temperature range of 100-350 °C no reaction was observed, therefore it was decided to limit the reaction study to the temperature range of 400 °C to 500 °C. The tested catalysts show very low propane conversions, the unmodified BN is more active (at 500 °C $C_{BN} = 2.8\% > C_{BNplasma} = 1.4\%$). The observations may be explained by the reduced specific surface area of BN_{plasma} compared to BN. However, the BN_{plasma} powder shows a much higher selectivity to propene (S = 57%) than the unmodified BN (S = 39%).

BN and BN_{plasma} were also used as carriers for heteropolyacids (HPA: $H_3PW_{12}O_{40}\times 28H_2O$ (HPW) and $H_3PMo_{12}O_{40}\times 36H_2O$ (HPMo)). The synthesis of HPA-BN catalysts with HPA content of 10 wt% was carried out by a wet impregnation method. HPA was applied to BN from ethanol solution at room temperature by stirring the obtained suspension for 2 h, and then the samples were dried at 50 ℃ for 24 h. The obtained catalysts were subjected to activity tests in the dehydration reaction of n-butanol (BuOH) in a gas flow fixed-bed reactor. Before starting the measurements, the catalysts were activated at 90 ℃ for 30 min in a He flow. The measurements were carried out in the temperature range of 90-230 ℃, with on-line detection of the reactants using a gas spectrometer with a TCD detector.

The studied systems were active in the dehydration reaction of n-butanol. Systems containing HPW showed higher BuOH conversions than those containing HPMo. The main reaction product is cis-2-butene. In the process, the catalysts containing HPW showed higher yields to cis-2-butene (Y_{max} = 75%) than HPMo (Y_{max} = 23%). HPW-BN catalysts were more active than HPW-BN_{plasma} in the temperature range of 90-230 ℃ (as shown by higher BuOH conversion), while HPMo-BN and HPMo-BN_{plasma} systems showed similar activity in the entire range of the tested temperatures.

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Physics and Chemistry of Surfaces and Nanostructures

5. Fluorescent metal nanoclusters monolayer of controlled structure and electrokinetic properties prepared on surfaces of colloidal carriers

(Associate professor Magdalena Oćwieja, Marta Sadowska PhD, Małgorzata Nattich-Rak DSc, Katarzyna Kusak Eng., Monika Wasilewska PhD, Paulina Żeliszewska PhD, Julia Maciejewska-Prończuk PhD, Dorota Duraczyńska PhD, Aleksandra Pacuła DSc, Alicja Michalik PhD, Piotr Smoleń MSc, Oliwia Kowalska MSc)

Fluorescent metal nanoclusters (Me NCs), consisting of several to a few dozen metal atoms and synthesized on protein matrices, exhibit molecular properties such as discrete electronic states, excellent photostability, high emission rates, and low toxicity compared to their nanoparticle counterparts. Despite extensive research on the use of nanoclusters as fluorescent probes, there is a lack of data in the literature regarding the mechanisms of their immobilization on interfacial surfaces. It is assumed that the creation of fluorescent nanocluster layers on the surfaces of colloidal carriers could significantly expand their application spectrum. Therefore, the aim of the research was to determine and describe the processes of depositing fluorescent metal nanoclusters onto the surfaces of colloidal carriers, such as silica and polystyrene particles.

During the reported period, the research focused, among other things, on the optimization of methods for obtaining stable silver and copper nanocluster hydrosols on lysozyme (LIZ) and human (HSA) and bovine (BSA) albumin matrices. Furthermore, attention was directed towards determining the luminescent properties of gold nanoclusters obtained on the aforementioned protein matrices, and research on the mechanisms of gold nanocluster deposition on the surfaces of colloidal carriers was continued.

During the conducted research, stable hydrosols of the following nanoclusters were obtained: HSA-Ag NCs, HSA-Cu NCs, BSA-Ag NCs, BSA-Cu NCs, LIZ-Ag NCs, LIZ-Cu NCs. The physicochemical characterization of HSA-Ag NCs and HSA-Cu NCs, performed using dynamic light scattering and electrophoretic light scattering techniques under controlled pH, ionic strength, and temperature conditions, showed that both types of nanoclusters have similar stability ranges and comparable electrokinetic properties, which also correlated well with the properties of HSA-Au NCs. All types of nanoclusters were stable in the pH range of 4.0 > pH > 5.8 (for ionic strength not higher than 0.15 M) and had an isoelectric point at pH 4.5.

The spectroscopic results obtained for gold nanoclusters on HSA, BSA, and LIZ matrices showed that the average fluorescence lifetimes and quantum yield of their fluorescence strongly depend on pH, ionic strength, and protein type. For example, at an ionic strength of 0.001 M, BSA-Au NCs exhibited an average fluorescence lifetime of 200 ns and 90 ns at pH 3.5 and 9.0, respectively, while for LIZ-Au NCs, fluorescence lifetimes of 120 ns and 90 ns were determined at pH 3.5 and 9.0. The fluorescence quantum yield ranged from 5% to 9%. The highest value was observed for LIZ-Au NCs at the isoelectric point. Additionally, circular dichroism (CD) spectroscopy studies were conducted, which allowed the identification of the contributions of individual protein structures in the obtained gold nanoclusters.

As part of further research, work was undertaken to develop a method for preparing LYZ-Au NCs layers on the surface of colloidal silica with an average size of 800±12 nm. For this purpose, a physicochemical characterization of silica dispersed in electrolyte solutions was conducted. Electrophoretic mobility and hydrodynamic radius dependencies as a function of pH and ionic strength were determined. SEM imaging was also performed. On the characterized silica, LYZ-Au NCs monolayers were formed, and the zeta potential of silica as a function of nanocluster concentration (1-5 $\mathrm{mgL^{-1}}$) was measured. Measurements were carried out for three ionic strength values (0.001, 0.01, 0.15 M NaCl). Using confocal microscopy imaging and spectrofluorometric measurements, it was demonstrated that the deposited monolayers of nanoclusters retained their fluorescent properties. This confirmed the production of fluorescent colloidal particles with sizes significantly larger than those of the unbound LYZ-Au NCs.

6. Physicochemical and mechanical properties of "bio" systems

(Maria Morga PhD, Associate professor Jakub Barbasz, Associate professor Piotr Batys, Leszek Krzemień PhD, Aneta Michna Dsc, Agata Pomorska PhD)

A detailed modeling of intermediate states in the process of lysozyme protein aggregation induced by the aggregating agent heparin was conducted under conditions corresponding to the native environment. The analysis included the identification of key stages of the aggregation process, such as initiation, nucleation, and the development of a methodology to study the growth of larger protein structures. A comprehensive methodology was developed to accurately determine process parameters, such as intermolecular interaction energies, bond formation dynamics, and protein conformational changes, based on the results obtained from molecular dynamics simulations. The modeling also enabled the identification of key amino acid residues involved in the aggregation process and the evaluation of heparin's role in stabilizing intermediate states. These findings may serve as a basis for further research into the molecular mechanisms of protein aggregation and the design of strategies to prevent pathological aggregation processes.

7. Structural, electronic and dynamics properties of surface and nanostructures studied with microscopic and spectroscopic techniques under ultra-high vacuum conditions.

(Professor Nika Spiridis, Professor Józef Korecki, Jacek Gurgul DSc, Kinga Freindl PhD Eng., Mariusz Gackowski PhD, Ewa Madej PhD Eng., Anna Miłaczewska-Kręgiel PhD, Ewa Młyńczak DSc, Bogna Napruszewska MSc, Robert Socha PhD, Dorota Wilgocka-Ślęzak PhD Eng., Bohdana Blyzniuk MSc, Natalia Kwiatek MSc, Małgorzata Nattich-Rak DSc)

Studies on the adsorption and surface morphology of organic thin films deposited on Ag(100) were conducted in collaboration with the University of Wrocław and the Institute of Experimental Physics at Johannes Kepler University in Linz. The structural properties of cobalt phthalocyanine (CoPc), fluorinated copper phthalocyanine (F16CuPc), and bimolecular films (composed of CoPc and F16CuPc) adsorbed on the Ag(100) surface at room temperature and subsequently annealed at 470 K were investigated. CoPc coverage at the monolayer level (ML) reduces the work function of the silver surface, while F16CuPc increases it. Due to the electrostatic interaction between the negatively charged fluorine in F16CuPc and the positively charged hydrogen in CoPc, the first monolayer of the mixed phase, in a 1:1 ratio, forms a chessboard-like superstructure. The formation of domains with opposite chirality was observed, which is associated with the specific adsorption of molecules on the Ag(100) surface. It was demonstrated that the application of the μLEED mode of the LEEM microscope to the diluted 2D molecular gas formed by about 0.5 ML of F16CuPc on the Ag(100) surface enables direct imaging of the molecular structure factor, thereby identifying the adsorption geometry of the molecule.

Research on ultrathin Sn films on the Pt(111) substrate continued. The initial stages of Sn growth on Pt(111) were studied using scanning tunneling microscopy (STM). Sn was deposited on Pt(111) at 27°C. The morphology and layer ordering were analyzed for the (√3x√3)R30° and c(4x2) superstructures, corresponding to 0.33 ML and 0.5 ML coverages, which had been identified in earlier studies using low-energy electron microscopy (LEEM). Studies were also conducted for intermediate coverages, where the superstructures were not fully developed or were a mixture of superstructures. A temporal instability in the Sn layers, dependent on coverage, was observed, most prominently for the coverage of about 0.5 ML, where the formation of areas with (√7x√7)R19.1° reconstruction was observed over time.

Research on spintronic systems based on 3d metal oxides continued. For the epitaxial systems Co/magnetite/Pt/MgO(111) and Co/hematite/Pt/MgO(111), measurements from the previous year, taken using the PEEM microscope on the DEMETER line and at the XAS station on the PIRX line at the Solaris National Synchrotron Radiation Centre, were analyzed. An analysis of the chemical state of the Co/iron oxide interfaces and the correlation between the magnetic structures of the sublayers was performed.

As in previous years, several dozen XPS analyses were conducted to determine the chemical state and oxidation degree of catalytic materials and other functional materials. In collaboration with the Solaris National Synchrotron Radiation Centre, the "Surface Nanostructures" group participated in the maintenance of the instrumentation infrastructure and in measurements on the DEMETER research line. As part of this activity, the group members contributed to several research projects, both their own and those of external users from Poland and abroad. As part of our own research, the epitaxial system Co/magnetite/MgO(111) and the adsorption of oxygen on W(110) were investigated.

8. Computer modeling of degraded plastic surfaces using reactive molecular dynamics

(Professor Tomasz Pańczyk, Paweł Wolski PhD)

Plastic waste poses an increasingly serious threat to the environment, particularly to marine organisms. Under the influence of ultraviolet radiation and mechanical forces, plastic waste undergoes significant chemical transformations and fragmentation, leading to the formation of micro- and nanoparticles referred to as microplastics and nanoplastics. The surfaces of these particles differ significantly in chemical composition from the surfaces of the original materials, posing a threat to living organisms, including humans. Therefore, the goal of the conducted research was to theoretically predict the surface chemistry of nanoparticles derived from plastic materials, primarily polyethylene, polyethylene terephthalate (PET), polystyrene (PS), and polypropylene (PP). Due to the need to analyze chemical reactions and their products, reactive force fields and techniques enabling the activation of chemical reactions were employed. For this purpose, the ReaxFF force field, as well as mechanical cleaving and shock compression of the material, were used.

The obtained results enabled the development of an innovative methodology for modeling degraded surfaces of plastic materials resulting from the impact of mechanical forces and the activation of reactions within the material due to UV radiation.

Using this methodology, the degradation processes of polyethylene terephthalate (PET) were studied. Simulations employed the ReaxFF reactive force field designed for CHO (carbon-hydrogen-oxygen) systems in the "combustion" branch. PET samples were subjected to mechanical cleaving to create PET surfaces. These surfaces were then analyzed to evaluate their chemical composition, as well as the quantity and types of functional groups formed on them. Benzene radicals and dioxolane residues were identified as the most prevalent on these surfaces. Additionally, the samples were subjected to shock compression to induce more extensive chemical transformations resembling naturally degraded nanoplastics. This process led to the discovery of various bonds within the material volume, many of which appeared as new chemical bonds or free radicals on the surfaces. A key observation was the significant release of carbon dioxide during both mechanical fragmentation and shock compression of PET samples. Another important finding was that chemically degraded PET surfaces differ significantly from the original material.

Analogous studies of polypropylene (PP) and polystyrene (PS) concluded that mechanical cutting of these materials results in surfaces containing products of mechanochemical bond breaking. It was observed that both polymers fracture within the backbone, generating -CH2· and -CH·- radicals. The average surface densities of these radicals were estimated at 0.0415 Å^{-2} for PS and 0.0289 Å^{-2} for PP. Additionally, a small production of light hydrocarbons, such as cyclopropylbenzene and propene, was noted. Shock compression applied to PS samples revealed that the primary degradation reaction involves the fusion of two benzene rings, leading to their partial dearomatization. This reaction was repeated, resulting in multiple connections of this type in the form of clusters and chains, and under more extreme conditions, even 3D structures of this type. A much less likely pathway involved the linkage of a benzene ring with an aliphatic carbon atom in the main chain.

The obtained results provided a better understanding of possible degradation pathways for some of the most widely used plastic materials. They also opened opportunities for analyzing and evaluating degradation products of other materials.

9. Carbohydrate molecules under the influence of external mechanical forces: theoretical calculations

(Professor Wojciech Płaziński, Agnieszka Brzyska PhD, Valery Lutsyk MSc)

In the first part of the study, the mechanism of conformational changes in mono-, di-, and oligomers forming the chains of natural polysaccharides, such as alginates, under the influence of external, stretching mechanical forces was considered. Such transitions occur in AFM experiments, and understanding the observed dependencies (force vs. chain extension) as a function of the type of polysaccharide being studied can serve as a tool for quantitative analysis of chain composition in the case of homopolysaccharides, as well as for exploring less-well-understood conformational degrees of freedom (e.g., changes in the conformation of pyranose rings). Advanced theoretical methods were employed in the study, including quantum mechanics methodology (e.g., the EGO method and molecular dynamics simulations using hybrid QM/MM potentials). The results showed a strong dependence of the force vs. extension profiles on the type of sugar unit considered (mannuronate or guluronate), which is related to the topology of the glycosidic linkages attached to the respective monomer. The more complex nature of the energy profile for the stretching of the guluronate monomer is associated with the occurrence of a series of ring distortion processes, which are absent in the case of mannuronate. Furthermore, for chains with a heterogeneous composition, the progression of such stretching profiles depends on the ratio of mannuronate to guluronate units, which may, in the future, allow for quantitative analysis of the chain composition based on AFM data. The results were published in the paper [10.1016/j.carres.2024.109294].

In the second part of the study, the problem of reconstructing NMR spectroscopic data (mainly chemical shifts) for a series of monosaccharides was addressed, using a method previously developed by our team: [10.1039/C7CP02920A]. By applying a combination of classical molecular dynamics simulations and quantum mechanical optimizations for a rationally selected sub-group of molecular configurations, very good agreement with experimental data was obtained for a group of eleven monosaccharides. Additionally, it was found that empirical relationships could be derived between the chemical shifts of atoms forming the rotating substituents of monosaccharides (e.g., the hydroxy-methyl group of aldohexopyranose) and the conformation of these substituents, analogous to Karplus's equation. This enabled the estimation of the population of rotamers of the hydroxy-methyl group based solely on the chemical shift values for the C6, H6R, and H6S atoms. This discovery opens new possibilities for using the set of chemical shift values not only as data describing the chemical structure but also the dynamic conformational changes of carbohydrate molecules.

10. Determination of mechanisms of synthesis, structure, and transport parameters of supported colloidal particle multilayers

(Associate professor Paweł Weroński)

Micas are a group of aluminosilicates with remarkable properties and numerous applications. In scientific research, this mineral is used, among other things, due to its excellent cleavage, which allows for the easy creation of atomically smooth surfaces. This property makes micas frequently used as model substrates for studying the processes of colloidal particle adsorption at the liquid/solid interface. One of the most important parameters determining these processes is the electric potential of the mica surface, usually approximated by the zeta potential. This depends on the pH of the solution from which the particles are deposited, its composition, and the ionic strength. The relationship between the surface potential and the type of ions used allows for the control of this potential, at a fixed pH and ionic strength, through the use of mixtures of different electrolytes. So far, this method has not been widely used due to the lack of a theoretical description of the dependence of the mica zeta potential on the electrolyte composition.

In the course of our research, we developed a generalized theoretical model quantitatively describing the dependence of the mica zeta potential on the composition of a mixture of two monovalent electrolytes. In our studies, we applied the classical Gouy-Chapman-Stern-Grahame (GCSG) approximation of the double electric layer, previously extended to describe the mica zeta potential in single-component electrolytes. We expanded this model to mixtures containing two monovalent cations, neglecting the difference between their ionic radii. This approach is equivalent to including the specific properties of the ions in the theoretical model solely through the equilibrium constants of their exchange reactions with protons present on the mica surface. These constants, along with the proton adsorption constant, were determined from experiments conducted on single-component systems using the classical GCSG model. The obtained parameters were then used for modeling binary systems composed of KCl and NaCl electrolytes with a total concentration of 0.1 mol/dm^3. To this end, we developed a numerical calculation algorithm allowing the determination of the mica zeta potential in electrolytes of varying composition. Subsequently, we performed a series of calculations for the studied systems. The theoretical results were compared with experimental data.

The conducted research allowed for the evaluation of the developed model's utility and the limits of its applicability. Theoretical predictions were qualitatively consistent with experimental results. However, the calculated values were associated with significant errors compared to the measured values, reaching several tens of percent. In particular, it was found that the generalized GCSG model allows for a correct description of the mica zeta potential in electrolytes with relatively low concentrations, optimally up to 0.01 mol/dm^3. This is a consequence of the limitations of the Poisson-Boltzmann equation, based on the assumption of the equality of the mean-force potential and mean potential of the ion in the electrolyte, which is only valid for dilute electrolytes. Therefore, further experimental and theoretical studies using mixtures of electrolytes with lower concentrations are planned.

11. Theranostic nanocarriers of anticancer drugs

(Associate professor Krzysztof Szczepanowicz, Tomasz Kruk PhD, Joanna Odrobińska-Baliś PhD, Anna Pajor-Świerzy PhD, Joanna Szechyńska MSc)

Despite very significant progress in cancer research, many people still die due to the lack of effective methods of diagnosing and treating cancer. Late diagnosis and multi-drug resistance of neoplastic cells are serious clinical problems. The main problem is the low selectivity of chemotherapeutic agents, which mainly results from systemic administration as well as poor solubility of chemotherapeutics in an aqueous medium (there are mainly hydrophobic substances). Therefore, it seems reasonable to use carriers of anti-cancer drugs to improve the biodistribution of therapeutic substances in the system, and thus increase their therapeutic index. Nanotechnology creates new perspectives, as it enables the transport of drugs to the target, and nano-drug preparations may have unique pharmacokinetic but also pharmacodynamic properties.

In 2024, we were focused on developing theranostic nanocarriers for targeted anticancer therapy. The theranostic nanocarriers of anticancer drugs were formed by the emulsification solvent evaporation method, while the sequential adsorption of charged nanoobjects method (called layer-by-layer) was used to functionalize prepared nanocarriers forming core@shell types nanocarriers. The anticancer drugs: staurosporin, salinomycin and docetaxel were selected for encapsulation into core@shell nanocarriers formed with poly-L-lysine, rodamine labeled poly-L-lysine and poly-L-glutamic acid (PGA). The size of prepared nanocarriers ranged between 100-200 nm. The properties of developed nanocarriers were optimized for passive targeting based on the EPR effect.

At the next stages of the research, the effects of encapsulated anticancer drugs and empty nanocarriers on the viability of the selected cell lines will be examined. Moreover, the ability to monitor them by optical imaging (OI) will be investigated.

12.Kinetics of coalescence in water-in-water emulsions

(Professor Jan Zawała, Andrzej Baliś PhD Eng., Dominik Kosior PhD, Georgi Gochev DSc, Agata Wiertel-Pochopień PhD Eng., Łukasz Witkowski MSc, Klaudia Zaręba MSc)

The goal of the study was to determine the kinetics of droplet coalescence in biphasic polymer systems, known as Aqueous Two-Phase Systems (ATPS), which undergo spontaneous separation under specific proportions. ATPS are highly intriguing systems composed of two immiscible aqueous solutions in thermodynamic equilibrium. These systems are of significant practical interest (e.g., in extraction, separation, purification, and enrichment processes of cellular organelles, proteins, carbon nanotubes, and metal ions, as well as in controlled drug delivery applications). Their primary advantage lies in their high biocompatibility, as they lack the organic phase present in conventional oil-water (O/W) emulsions. The main challenge in utilizing ATPS, as well as emulsions and capsules derived from them for targeted drug delivery, is the low stability of dispersions due to extremely low interfacial tension. The research efforts outlined in this task aim to identify effective stabilizers for ATPS systems, which would significantly expand their application range and functionality.

During the reporting period, measurements were conducted using a single ATPS system comprising aqueous solutions of polyethylene glycol (PEG) with a molecular weight of 10 kDa (7.9 wt%) and dextran (DEX) with a molecular weight of 75 kDa (7.9 wt%) supplemented with microgel nanoparticles of various sizes derived from β-lactoglobulin (BLG). Based on these substances, transient PEG-in-DEX emulsions stabilized by BLG particles were obtained, and the rate of phase separation (emulsion breakdown) was investigated. The effect of pH (ranging from 2 to 6) on emulsion stability was studied for particles of constant size (64 nm). The most stable emulsion was obtained at pH 3. Subsequent experiments were conducted with emulsions at pH 3 using BLG particles at a concentration of 0.1 wt% and varying sizes (40 to 190 nm). It was demonstrated that the maximum emulsion stability (on the order of an hour) was achieved with BLG particles of intermediate size (70–80 nm). It was shown that the stability of the ATPS system stabilized by BLG particles depends critically on the particle size and the pH conditions under which the emulsion is formed. The most stable emulsions were obtained at pH 3 (see Fig. 1) and with particle sizes of 80 nm. Under these conditions, BLG particles preferentially accumulate in the interfacial region (between the two aqueous phases), thereby counteracting droplet coalescence. In broader terms, generalizing this correlation requires studying various ATPS systems and particles with different surface properties.

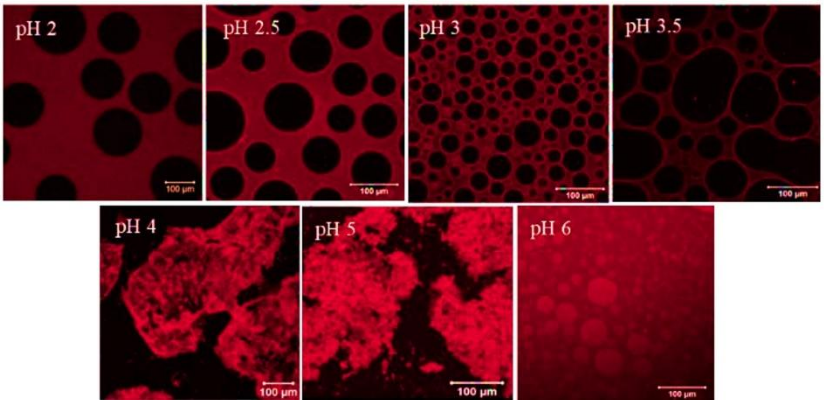


Fig. 1. Representative images obtained using confocal laser scanning microscopy for PEG-in-DEX emulsions/BLG particles (64 nm diameter, 0.2 wt% BLG) at different pH values. At pH = 3, the degree of dispersion was the highest – accumulation of BLG particles in the interfacial region (at the edges of the droplets) is clearly visible.

13.Natural and modified polysaccharides as stabilizers of surface films, foams and emulsions

(Ewelina Jarek PhD, Lilianna Szyk-Warszyńska PhD, Professor Piotr Warszyński, Marzena Noworyta MSc, Marcel Krzan DSc, Marta Kolasińska Sojka DSc)

As part of a statutory task, we studied the properties of emulsions: aqueous solutions of polysaccharides (chitosan-polycation and furceleran-polyanion) with plant essential oils (oregano, thyme, rosemary and lemongrass). Studies have shown that polysaccharides can contribute to the electrostatic stabilisation of emulsions through adsorption at the interface, and also counteract coalescence by increasing solution viscosity in the presence of polysaccharides. An in-depth analysis of the IR spectra using DFT methods to calculate theoretical spectra for the main components of the commercial oils confirmed that these are carvacrol for oregano, thymol for thyme, 1,8-cyneol for rosemary and citral for lemongrass oil, respectively. In the case of carvacrol and thymol, we can see a sharp band ~ 3630 cm^{-1} in the computational spectra (Figs. 1a. and 1b.), since the calculations were conducted for isolated molecules. free hydroxyl groups only occur in the vapor phase or in very dilute solutions in non-polar solvents. Therefore, in experimental spectra for oregano and thyme oils, we observe a strong, broad band in the 3600-3100 cm^{-1} range, as results of OH groups presence involved in intermolecular interaction via hydrogen bonds. In the case of lemongrass oil and rosemary, weak bands in this region do not exclude the presence of OH groups. Otherwise, the experimental spectra are well reflected by the computational ones obtained for the main components of essential oils. The appearance of bands in the region 1700-1740 cm^{-1} may suggest the presence of some organic acids in the essential oils, which may interact electrostatically with policataion as chitosan is. We have prepared the paper „Nanoformulation of natural essential oils, bio-polysaccharides (furcellaran, chitosan) and antimicrobial peptides (RW4, LL37) for application in the food industry – formation, stability and properties". Moreover the stability of complex emulsions containing polysaccharides and medium chain triglycerides (MCT) as oil phase has been investigated.

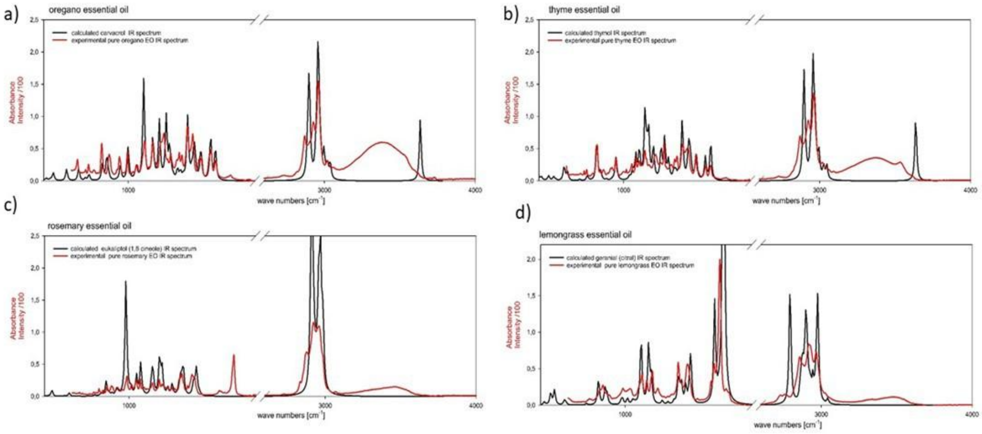


Fig.1 Comparison between experimental IR spectra of pure essential oil and theoretically calculated, by using DFT method, ones for main component of essential oils molecules a) carvacrol for oregano EO, b) thymol for thyme essential oil, c) 1,8-cineole for rosemary EO, d) citral for lemongrass EO

14. Influence of severe plastic deformation of Mg on the properties of phosphate layers

(Konrad Skowron PhD Eng., Grzegorz Mordarski PhD Eng., Małgorzata Zimowska DSc, Michał Mosiałek DSc)

The aim of the work conducted in 2024 was to continue the research initiated in 2023 on the effect of severe plastic deformation of Mg alloys on the physicochemical properties of conversion coatings deposited on them. The influence of applied mechanical treatment on Mg microstructure was studied. These investigations were correlated with electrochemical corrosion studies of Mg alloys coated with phosphate conversion layers.

The deformation process was carried out with surface mechanical attrition treatment (SMAT) with different parameters for AZ series Mg alloys to obtain surface structures with different morphologies, which were characterized using available physicochemical methods. Scanning electron microscopy, X-ray diffraction, X-ray photoelectron spectroscopy, and infrared spectroscopy were utilized. The protective properties of the obtained materials were examined using the potentiodynamic polarization and immersion tests in NaCl solutions.

The influence of severe deformation on the properties of AZ31 and AZ91-T6 magnesium alloys was studied. Electrochemical studies of alloys were conducted in NaCl solutions. Conversion layers from phosphate baths were deposited on the surfaces prepared in this manner. Long-term hydrogen evolution tests in NaCl solutions were performed for alloys before and after SMAT treatment. The hydrogen evolution tests showed that samples subjected to deformation exhibited lower hydrogen evolution compared to the reference samples.

The process of severe plastic deformation influences the corrosion behavior of the studied magnesium alloy, making it more reactive at initial stages of exposure to corrosive environments, which in turn accelerates the formation of a dense surface layer of corrosion products upon immersion in NaCl solutions. This, in turn, leads to improved corrosion resistance during the initial hours of immersion. The use of the SMAT method affects the quality of deposited conversion layers.

15.Molecular aspects of protein conformational stability in the context of the formation of amyloid superstructures

(Professor Barbara Jachimska, Agnieszka Kamińska PhD Eng., Magdalena Goncerz MSc Eng., Kamil Rakowski MSc, Łukasz Luśtyk MSc Eng.)

The study of the conformational stability of protein layers at the solid-liquid interface is crucial for maintaining the protein's biological activity and understanding the interactions of adsorbed proteins with lipid membranes. Changes in protein structure can lead to the development of many diseases, including neurodegenerative diseases. The main objective of this study was to analyze the structure of lysozyme adsorbed on the gold surface and lipid membrane under variable environmental conditions. Physicochemical properties of lysozyme, such as size and zeta potential, were measured using DLS. Quartz crystal microbalance with energy dispersion (QCM-D), multiparameter surface plasmon resonance (MP-SPR), and Fourier transform infrared spectroscopy (FT-IR) were used to determine the adsorption efficiency of lysozyme and hydration of the lysozyme layer formed at the interface. The secondary structure of lysozyme was analyzed using circular dichroism (CD). In addition, FT-IR spectra of amide I were used to monitor the structural stability on the adsorption surface. XPS studies were performed for lysozyme adsorbed on the gold surface and lipid membrane.

MP-SPR and QCM-D studies showed that during the formation of a monolayer on the gold surface, the orientation of molecules changes with increasing surface coverage. A higher hydration level of the adsorbed protein layer (78-90%) was obtained for lysozyme adsorbed at pH 4.0 compared to pH 7.5 (58-72%). The structural stability of lysozyme on the surface was controlled by deconvolution of the Amide I band, which responds to conformational changes within the protein structure.

In addition, different changes in the secondary structure of lysozyme were found on the gold surface and the lipid membrane. The occurrence of antiparallel β-sheets is observed on the gold surface and may indicate the beginning of the protein misfolding process and its destabilization. Lysozyme adsorption on the lipid membrane surface leads to an increase in the content of disordered structures.

The determined kinetic rate constants (ka, kd) and equilibrium dissociation constants indicate a higher affinity of lysozyme to the gold surface for low pH values (4.0). In turn, the adsorption of lysozyme on the surface of the lipid membrane is less sensitive to differences in the pH of the environment, and the adsorption efficiency is at a similar level. Based on the obtained values of the K_D constant, the affinity of lysozyme molecules to the membrane surface was higher than the affinity to the gold surface, regardless of the environmental conditions. Based on the above studies, it can be concluded that in the case of the lipid membrane, the contribution of hydrophobic interactions to the stability of the protein layers is visible.

Physics and Chemistry in Cultural Heritage Protection

16. Quantitative assessment of the threat to historic objects caused by environmental conditions in their surroundings

(Professor Łukasz Bratasz, Marcin Strojecki PhD, Nefeli Avgerou, Sergii Antropov PhD)

The monitored objects were of different sizes, building structures, heating strategies and systems, and liturgies, because the research included not only Roman Catholic churches but also Orthodox churches. In each object, the measurement system consisted of at least two dust concentration sensors in two particle size fractions - fine (0.3 - 1 μm) and coarse (above 1 μm). The sensors were placed in different locations in the vertical profile next to air temperature and relative humidity sensors, an air velocity and direction sensor, and a carbon dioxide concentration sensor.

The collected data, taking into account the significant variability of dust concentration outside and the different patterns of use of individual buildings, were used to determine the parameters characterizing the monitored interiors, such as the air exchange rate (AER), penetration (P) and dust deposition rate (k). As a result of the analysis, it was determined that:

- the AER value varies within wide limits between 0.08 per hour (a large medieval brick church with small windows) and 1.2 per hour (a small wooden church with a relatively open structure).
- the P values take on unfavourable, relatively high values between 0.7 and 0.9 for particles with a diameter between 0.3 and 1 μm (fine fraction) and from 0.16 to 0.75 for particles with a diameter above 1 μm (coarse fraction).

The determined k values were used to calculate the key parameter influencing the soiling rate, which is the velocity of particle deposition on architectural surfaces and equipment vd. The value of vd, depending on the particle size, varies between 0.7 and 4.7·10-5 m/s and 1.6 and 7.8·10-5 m/s, respectively, for fine and coarse particles.

The obtained vd values and calculated average concentrations allowed for estimating the composition of individual sources in the total number of dust particles that can settle on a single internal surface during the year Ndep. Analysis of this synthetic indicator of soiling allows for comparing objects with each other in the context of internal and external sources in the cold and warm periods and determining the potential impact of heating or its absence.

The determined values of the settling rate show significant variability for both size modes and agree well with the literature data for rooms with relatively low air flow and significant roughness of the deposition surface. The impact of intensive heating strategies with increased average internal temperature on the increased particle deposition rate was also observed. The additional numerical analysis allows us to conclude that reducing the AER coefficient at a constant penetration coefficient, in the case of historic buildings such as the examined sacral buildings, has a limited effect on reducing the average concentration of suspended dust inside (less than 10%), and only smoothes out the fast fluctuations of the dust concentration inside.

Development grant

1. Enzymatic synthesis of biologically active regioselectively dehydrogenated pentacyclic triterpenes

ICSC PAS development project no. 1/GR/2024 [2024-2026]
(Agnieszka Wojtkiewicz PhD)

The project aims to develop a biocatalytic oxidative dehydrogenation method for novel pentacyclic triterpenes (TP) using an enzyme from the 3-ketosteroid dehydrogenase (KSTD) family. This work focuses on producing health-promoting compounds in quantities suitable for application studies and scaling the technology to a semi-technical level.

Significant progress was made in the first year. A bacterial culture was optimized using minimal medium at a 5 L scale, achieving a tenfold increase in biomass compared to LB medium. The process was successfully scaled up to 35 L. A rapid enzyme isolation method was developed, although the catalyst showed low stability during storage. Syntheses were performed at a 0.1 L scale for three new TP derivatives, yielding previously unreported products. Reaction monitoring methods (LC-MS and TLC) were established, and a purification station was set up. While progress was made, optimization of the purification method is ongoing. Two patent applications were prepared, and two grant proposals were submitted to NCS and FNP to support further research.

Plans for the second year include scaling up syntheses to 0.9 L, refining purification methods, conducting biological studies, depositing compounds in the Polish Chemical Library OPENSCREEN, finalizing patent applications, writing a publication, and seeking additional external funding.

2. Hybrid mineral composites obtained by heterocoagulation, as effective nanomaterials for CO_2 capture

ICSC PAS development project no. 2/GR/2024 [2024-2026]
(Małgorzata Zimowska DSc)

According to the Green Chemistry concept, when designing and conducting chemical processes, the key is to sustainably use resources, reduce the use and production of harmful materials and chemicals, and reduce greenhouse gas emissions. Efforts should therefore be made to use renewable raw materials and to carry out catalytic processes aimed at diminishingthe level of CO_2 in the atmosphere or its transformation into more desirable products in industrial and environmental applications.

This project aims to develop innovative bi-functionalised hybrid mineral composites (anionic-cationic mineral brushes) with a basic character, active in the capture and sequestration of CO_2 from flue gases. In this project, we focused on the preparation and modification of hydrotalcite precursors, their derivatives obtained by thermal treatment and hybrid, anionic-cationic mineral brushes generated on a phyllosilicate matrix (smectite), acting as a structure-forming platform. Our research focused on a detailed analysis of the structural modification of the mineral composites and its effect on the development of enhanced CO_2 sorption capacity.

In the first step, using a co-precipitation method, a series of anionic layered double hydroxides (LDH), with a hydrotalcite structure $[M^{2+}{}_{1-x}M^{3+}{}_x(OH)_2][An^-]_{x/n}\cdot mH_2O$, with compositions Mg-Al=2:1, 3:1, 5:1, differing in ageing time, degree of crystallinity and hydrotalcite grain size, controlled by the synthesis conditions, were obtained. The materials obtained were characterised using XRD, FTIR, SEM/EDS, and N_2 adsorption/desorption methods for structural-sorption correlation. Qualitative-quantitative studies of the generated basic centres were carried out using in situ spectroscopic methods with the CO_2 probe molecule and Temperature-Programmed CO_2 Desorption. The influence of the synthesis parameters on the nature of the generated basic centres their strength, CO_2 adsorption/desorption process kinetics and sorption capacity was investigated.

The synthesised precursors had a crystalline structure characteristic of hydrotalcite with R3m rhombohedral symmetry and crystallite sizes of 10, 20 and 30 nm. The crystallites of the resulting MgO oxide were in the range of 3-6 nm. FTIR spectroscopic studies of the anionic hydrotalcite-like precursors and their mixed oxides exhibited that CO_2 reacts with the O^{2-} and OH^- to form carbonate (CO_3^{2-}) and bicarbonate (HCO_3^-) ions. Carbonates and bicarbonates are formed at low surface coverages and there is no preference as to which O^{2-} or OH^- basic centres react with CO_2 first. Treatment in a vacuum affects the generation of free OH groups and the formation of carbonates and bicarbonates. Aging time and crystallinity affect the availability of the generated basic centres and the number of OH^- groups formed, of which only some (free OH^- groups) are basic enough to react with CO_2. Calcination increases the basicity of hydrotalcite, the O^{2-} centres have a higher strength than the OH^- groups. Calcined samples, derived mixed oxides, form more carbonates than their precursors, due to the greater number of available O^{2-} centres.

"Sonata Bis" Research Projects of the National Science Centre

1. Synergistic effects of mixed biosurfactants solutions in stability of liquid films under dynamic conditions - fundamental research with practical importance in flotation separation process

"Sonata Bis" research project NCN 2020/38/E/ST8/00173 [2021-2025]
(Project leader: Professor Jan Zawała)

The project is related to fundamental research on determination of mechanism of synergistic effects in solutions of chosen mixed flotation reagents. The experiments planned to be carried out are aimed to describe the reasons and conditions of synergism in relations to formation and stability of liquid films formed at various interfaces under dynamic conditions. New amino acid based surfactants and their mixtures with simple non-ionic surface-active substances as well as biodegradable microparticles were chosen for this purpose.

During the reporting period, concepts were developed for utilizing biopolymer particles of bacterial origin as potential reagents to enhance the efficiency of gravitational separation processes of valuable mineral particles. The primary advantage of the developed biopolymer particles is their biodegradability, which ensures no negative impact on the natural environment. The concept assumes that biopolymer particles (spheres) with sizes ranging from 70 to 500 nm will act as flocculating reagents, causing the agglomeration of fine-phase particles and increasing their size, thereby significantly accelerating sedimentation rates or enhancing recovery probabilities in flotation processes. Additionally, the adsorption of polymer microspheres onto mineral particles is expected to increase surface roughness and hydrophobicity, substantially improving the efficiency of forming flotation aggregates (bubble-mineral grain). A schematic mechanism of the biopolymer particles' action is shown in Fig. 1AB. The work conducted during the reporting period aimed to develop methodologies for synthesizing the particles and protocols for modifying their surfaces. Microscopic studies confirmed the effectiveness of the developed methods – the obtained particles exhibited a relatively high degree of polydispersity but, due to their positive surface charge, effectively adsorbed onto oppositely charged solid surfaces. Preliminary tests confirmed that the presence of polymer particles on the surfaces of glass plates (used as a model negatively charged surface) significantly reduced the time required for the formation of a three-phase contact (air bubble adhesion). Furthermore, the addition of a specific amount of particles to a suspension of quartz particles induced their agglomeration and faster sedimentation.

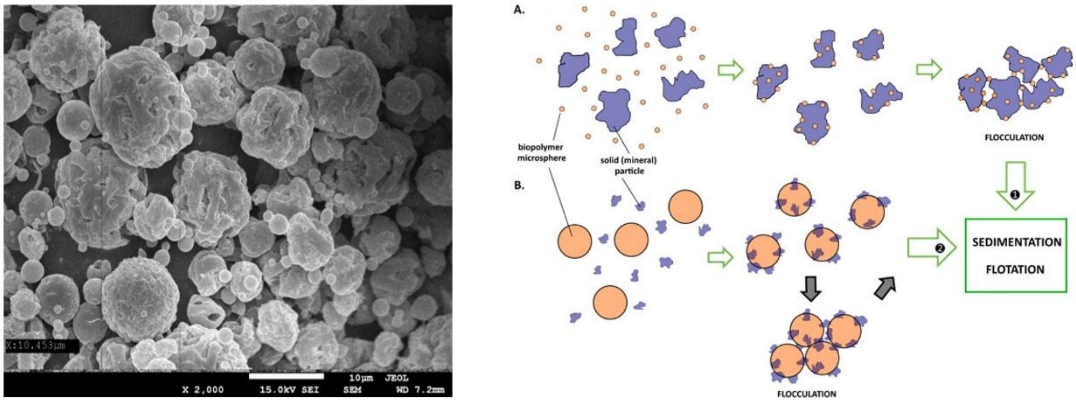


Fig. 1. An image of the obtained biopolymer particles (bacterial-origin polymer, image captured using Scanning Electron Microscopy) and a schematic mechanism illustrating the particles function as flocculating reagents, enhancing the efficiency of recovery through gravitational separation methods.

2. Topology meets magnetism: Sn-based compounds for magnetization-driven topological phase transitions (TopoTin)

TopoTin
Topology meets magnetism

"Sonata Bis" research project NCN 2022/46/E/ST3/00184 [2023-2028]
(Project leader: Ewa Młyńczak DSc)

The TopoTin project was launched on 15.03.2023. Since 01.10.2023, two PhD students of the Krakow Interdisciplinary Doctoral School (KISD) Ardra Surendran and Smibin Shaju have been involved in the project. The project is being implemented according to the plan, we are in the process of performing the tasks planned in the second year of the project. In our work, we use the molecular beam epitaxy method to prepare thin-film samples of intermetallic compounds containing Sn and Fe. Sample deposition is carried out in an ultrahigh vacuum system at IKiFP. Then, the samples are characterized in vacuum conditions using low-energy electron diffraction (LEED) and X-ray photoelectron spectroscopy (XPS). For some of the samples, we also carried out scanning tunneling microscopy (STM) measurements after transferring them in vacuum conditions using the so-called vacuum suitcase to the STM laboratory at IKiFP. In the project, we use access to the Solaris National Synchrotron Radiation Center, which is granted after the evaluation of applications by an international team of experts. In 2024, we carried out one experiment on the DEMETER line using the PEEM (photoemission electron microscope) end station and two experiments using the Phelix line (angle-resolved photoemission, ARPES). For some of the synchrotron experiments, we used samples transported in a vacuum case from our laboratory to Solaris. Further characterization was carried out using ex-situ methods, after covering the samples with a protective layer (including magnetic force microscopy MFM, X-ray diffraction XRD, Mossbauer spectroscopy CEMS, transmission electron microscopy TEM). In 2024, we focused on studies of the magnetic and electronic properties of Fe3Sn2 and FeSn layers prepared on a single-crystal Pt(111) substrate or on a thin Pt(111) layer pre-deposited on Al2O3(0001) crystals. Pt(111) layers were deposited using the sputtering method (in cooperation with the Institute of Physics of the Polish Academy of Sciences in Warsaw, Prof. Andrzej Wawro). We also continued research on the growth of ultrathin Sn layers on a Pt(111) substrate, using low-energy electron microscopy LEEM and scanning tunneling microscopy STM. The results of the work in the project in 2024 are a number of oral presentations given by dr. hab. Ewa Młyńczak (3 international conferences and 2 invited seminars: University of Wrocław and the Institute of Physics of the Polish Academy of Sciences in Warsaw) and 2 poster presentations at international conferences presented by PhD students Ardra Surendran and Smibin Shaju.

"Sonata" Research Projects of the National Science Centre

1. From a single molecule to smart material - understanding the polypeptide complexes formation and properties

"Sonata" research project NCN 2018/31/D/ST5/01866 [2019-2024]
(Project leader: Piotr Batys DSc Eng.)

A novel hybrid biomimetic design, i.e., a directed design process enabling the prediction of new polypeptide structures containing α-helical structures inspired by nature but with higher molecular stability, was developed. In the first stage, deep neural network models and multiscale computational modeling were used to select polypeptide sequences with the desired properties. MD modeling allowed to exclude some structures due to their low stability and to indicate the most promising ones. Then, twenty-five de novo designed polypeptide variants were genetically developed for recombinant expression in Escherichia coli. Of these, 15 variants were successfully expressed, of which 10 variants with the highest efficiency were selected for further studies. Further extensive atomic resolution MD simulations were performed for all 10 experimentally obtained polypeptides. Systems containing multiple polypeptide molecules were simulated at variable temperature to check the interactions between polypeptide molecules and their ability to phase separate (Fig. 1). Simulations showed that at elevated temperature, the polypeptides clustered, indicating the existence of a lower critical solution temperature (LCST). This feature is essential for regulating the phase behavior of polypeptide solutions and thus enables their further use in the production of functional materials with programmable properties.

Furthermore, potential of mean force (PMF) profiles were determined to determine the free energy of association of α-helices. The results showed that the ΔGassoc of α-helices was in the range of -5 to -75 kJ/mol, and therefore the association was energetically favorable for all the polypeptides studied. The free energy of association correlated with the lower critical solution temperature, indicating that the phase properties of such polypeptides can also be predicted by MD simulations.

The obtained polypeptides showed tunable, self-assembled structures and phase behavior, both in silico and in vitro. The most promising variants were used as programmable thermoresponsive injection matrices and permeability modulators for smart windows.

2. Improving the conductivity of printed patterns by optimizing the synthesis process and physicochemical properties of metal nanoparticles.

"Sonata" research project NCN 2020/39/D/ST5/01937 [2021-2024]
(Project leader: Anna Pajor-Świerzy PhD)

In recent years printed electronics have attracted much attraction due to the possibility of their use in many areas of our lives, from the production of intelligent packaging to innovative medical applications. In this process, inks and pastes based mainly on metal nanoparticles (NPs) are used, related to their specific and unique properties. The quality of printed electronic tracks depends on many parameters such as the properties of the ink/paste, the type of substrate, and printing methods. To obtain electronic circuits with satisfactory properties, these parameters must be optimized and compatible. In this context, the process of fabrication of printed conductive materials includes three stages: (1) preparation of the ink based on nanoparticles, (2) its deposition or printing using proper techniques, and (3) post-printing treatment of obtained coatings or circuits, e.g. drying and sintering. Silver nanoparticles have been most often used so far as a functional component of conductive nanomaterials. However, their high price and poor mechanical properties limit the use of Ag NPs on a wider industrial scale. Nickel, which has both good conductive and mechanical properties and is also a much cheaper metal, can successfully replace silver in the process of obtaining conductive materials. However, there is a problem of instability of its nanoparticles regarding the oxidation process. Therefore, it was proposed to cover the nickel core (Ni NPs) with a silver protective coating providing good resistance to oxidation of Ni NPs, resulting in the formation of core-shell structures (Ni-Ag NPs).

During the work carried out in 2024, a low-temperature method for the fabrication of conductive coatings based on Ni-Ag NPs was developed, combining the use of oxalic acid (OA) and the UV-Vis sintering process. Exposing the deposited coatings to OA and the UV-Vis irradiation process allowed obtaining materials with higher conductivity compared to those exposed only to oxalic acid or UV-Vis radiation. It was also noticed that the resistivity of the sintered layers depends on the thickness of the silver coating of Ni-Ag nanoparticles. Its value decreased with the increase in the thickness of the silver shell (Figure 1). The obtained results are promising regarding the use of the modified sintering method based on the low-temperature process to obtain conductive structures with future applications in the production of the "flexible" electronics.

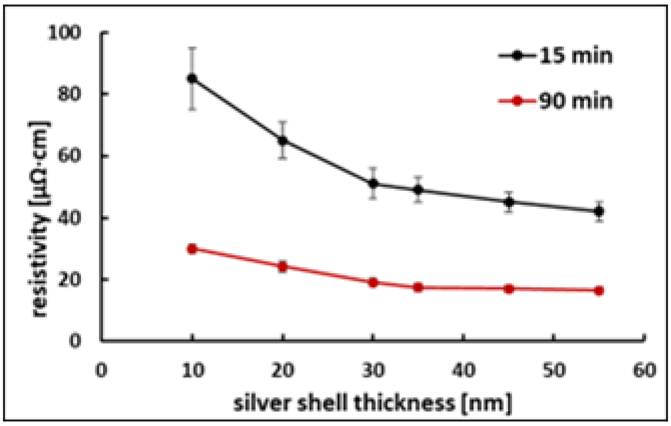


Fig. 1. The dependence of the resistivity of Ni@Ag NP-based coatings on the thickness of the silver layer after treatment with 1% OA (black curve) and after using the combination of two methods (1% OA and UV-Vis, red curve).

3. Nanohybrid systems containing graphene oxide and nanoparticles as functional components for a new type of biosensors

"Sonata" research project NCN 2022/47/D/ST5/01751 [2023-2026]
(Project leader: Tomasz Kruk PhD)

The aim of the project is focused on the development of a new type of highly sensitive biosensor with graphene oxide (GO) and/or GO hybrids with polymers and silver/copper nanoparticles, as supporting layer on the gold surface in the SPR biosensor. We postulate that due to several physicochemical advantages as well as optical properties, GO can be a good enhancer of SPR signal. Gold surface of the SPR sensor coated by GO will increase the sensitivity of SPR biosensor. Moreover, the biochemical properties of GO, as well as its versatile surface with various functional groups (epoxy, carboxyl, hydroxyl-group), enables facile covalent and noncovalent immobilization of biomolecules. The modification of graphene oxide with popular polymers such as poly-L-lysine, poly-L-arginine or polyethylene glycol will increase GO biocompatibility and will reduce its cytotoxicity. Furthermore, the addition of silver/copper nanoparticles will provide additional enhancement of the surface plasmon resonance signal. The functionality and sensitivity of the prepared immunosensors will be determined during a series of measurements of the interactions between antibodies and antigens.

The goal of the work in the period in question (01.01-31.12.2024) was to deposit a layer or layers of GO and GO hybrids with polyelectrolytes/metallic nanoparticles of copper (Cu)/silver (Ag) on the gold surface in a controlled and repeatable manner. Full physicochemical characterization was carried out and comparison of graphene oxide suspensions from various manufacturers (including Sigma Aldrich, PlasmaChem, Abalonyx). The physicochemical characterization of various GO suspensions and Ag/Cu nanoparticles suspensions obtained by chemical reduction methods were also carried out, including: size, zeta potential value, stability, composition and presence of different functional groups for GO using methods: DLS, UV-Vis, SEM and XPS. Additionally, using methods; AFM, SEM and ATR-FTIR determined the topology, coverage, surface roughness, structural and spectral properties of the tested systems. Quartz Crystal Microbalance with Dissipation monitoring (QCM-D), whose surface is covered with gold, and gold SPR sensors were used as model surfaces for deposition of graphene oxide/nanoparticles. The layer-by-layer (LbL) technique was used to form the layers, which is based on the adsorption of oppositely charged nano-objects using methods: immersion, spraying (air brushing) and spin coating. The deposited GO layers should be characterized by high surface coverage, high stability and homogeneity. Currently, research is aimed at optimizing GO deposition in a controlled, repeatable and homogeneous manner. The coverage of the gold surface with a GO layer (or layers) is verified, in order to select the most optimal method for obtaining a substrate with a layer of gold and graphene oxide for further work.

4. Interactions between bacterial liposaccharides and an “X” shaped peptide oligomer at interfaces: The X-factor in antimicrobial strategy?

"Sonata" research project NCN 2023/51/D/ST4/02300 [2024-2027]
(Project leader: Magdalena Włodek PhD)

Understanding the structure and interactions within the envelope of bacterial cells is essential for the development of new antimicrobial drugs. Gram-negative bacteria are particularly challenging targets due to the presence of an additional, highly asymmetric outer membrane composed of lipopolysaccharides (LPS). LPS is a molecule consisting of three structurally distinct parts: lipid A, a core oligosaccharide, and the O-antigen.

The main objective of the project is to investigate the interactions between LPS molecules and surfaces with different chemical properties, in particular, oligo-glycine (an X-shaped peptide). The results are expected to provide valuable insights into the potential use of the X-shaped peptide for capturing (and subsequently killing) Gram-negative bacteria on surfaces. This research will also contribute to a new scientific area of practical significance, paving the way for innovative approaches in biomedical applications.

In the initial phase, the focus will be on understanding the influence of solution conditions on LPS/Lipid A self-assembly and optimizing the preparation of LPS liposomes. LPS liposomes will be prepared using the standard extrusion method. Extrusion is a one-step process in which liposomes are formed by forcing lipid suspensions through filters with defined pore sizes.

In 2024, a setup for carrying out the extrusion of liposomes containing LPS/Lipid A and LPS was purchased and assembled, utilizing a pressure extruder combined with a heated circulating bath.

"Opus" Research Projects of the National Science Centre

1. New polysaccharide-based biomaterials as an effective platform for adsorption and release fibroblast growth factors: applications in diagnostics and treatment of diseases of affluence

"Opus" research project NCN 2018/31/B/ST8/03277 [2019-2023]
(Project leader: Aneta Michna DSc)

The main objectives of the project, completed in March 2024, were to develop an efficient method for producing biocompatible polyelectrolyte multilayers, with particular emphasis on polysaccharides, that enable the attachment and controlled release of two types of fibroblast growth factors (FGFs): FGF21 and FGF23. This development has potential applications in the treatment of civilization-related diseases.

Over the past year, the physicochemical characterization of FGF23 was carried out using molecular dynamics (MD) modeling and various advanced experimental techniques: multi-angle dynamic light scattering, streaming potential, reflectometry, and quartz crystal microbalance with dissipation monitoring (QCM-D). For the first time in the literature: molecular shape, cross-sectional area, the relationship between nominal charge and pH, and the isoelectric point (IEP), which was found to be 9.2, were determined. It was shown that the electrokinetic charge of FGF23 depends on pH, decreasing from +25e at pH 4 to -5e at pH 10. Additionally, FGF23 was found to reversibly adsorb onto negatively charged hard surfaces (oxidized silicon, mica), as demonstrated by reflectometric and gravimetric measurements (QCM-D). The maximum surface coverage of FGF23 on oxidized silicon was 2.8 g/m² in 0.01 M NaCl at pH 5.8, then decreased to 1.2 g/m² after controlled washing (reflectometric measurements). It was also shown that under the same conditions, FGF23 reversibly adsorbs onto mica, reducing the substrate's negative potential from -60 mV to -20 mV.

The completed project contributed to the development of a research methodology enabling the creation of new biomaterials as effective scaffolds for adsorption and controlled release of fibroblast growth factors (FGFs). The conducted studies demonstrated the central role of electrostatic interactions in the binding processes of proteins to biomaterials, which is significant for the development of the field, given the lack of experimental data in the literature obtained for model systems under in situ conditions, supported by theoretical analysis. In addition to its cognitive significance, the acquired knowledge will enable the future design of new materials with desirable properties, such as enhancing cell proliferation and survival, biocidal coatings, and biosensors for selective capture and binding of specific types of proteins from blood serum.

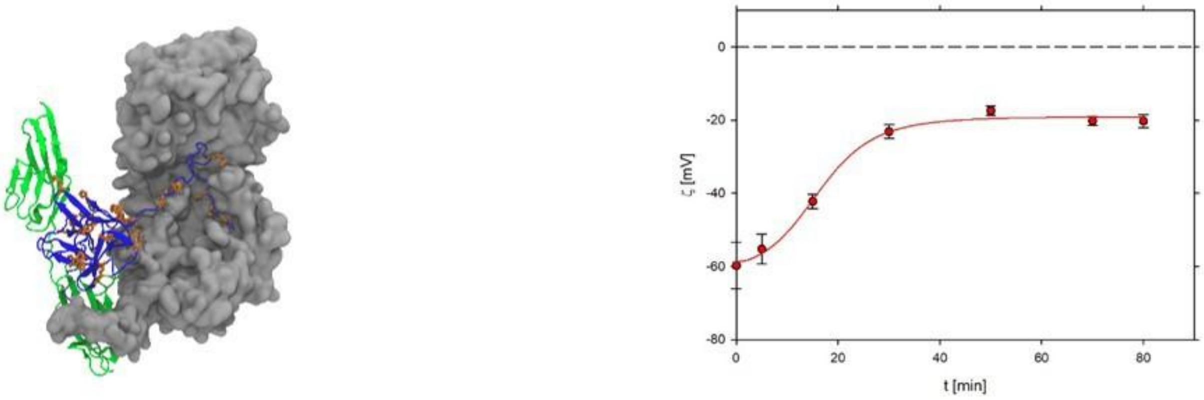


Fig. 1 a) FGF23 interacts with klotho protein (numerical calculations using molecular dynamics performed by Ph. D., D.Sc. Piotr Batys, prof. of the Jerzy Haber ICSC, PAS), b) The dependence of the zeta potential of FGF23 on mica vs. adsorption time. Measurements were conducted at an ionic strength of 0.01 M NaCl, pH 5.8 (streaming potential measurements)

2. Novel composites of smectite minerals and TiO_2 nanoparticles prepared by inverse microemulsion method for photocatalytic applications

"Opus" research project NCN 2018/31/B/ST5/03292 [2019-2024]
(Project leader: Professor Ewa Serwicka-Bahranowska)

The research carried out within the framework of the project, which ended in 2024, was aimed at designing and synthesizing a new type of composite photocatalysts obtained by integrating two synthetic procedures: one involving the formation of TiO_2 particles in an inverted microemulsion, i.e. in water nanodroplets dispersed in an organic liquid, and the other involving the delamination of clay minerals. The result was a new type of TiO_2/smectite composites, consisting of TiO_2 nanoparticles immobilized between exfoliated layers of smectite. Various modifications of montmorillonite, the most popular natural smectite, and laponite, a synthetic smectite with the widest spectrum of industrial applications, were used as TiO_2 carriers. The newly developed composite materials have been tested as photocatalysts for treating water from organic pollutants, mainly those that enter sewers and water bodies in large quantities, such as water-soluble dyes and humic acids. The activity of the composites in photodegrading both types of pollutants was very high, and the best of the composites outperformed the photoactivity of commercial TiO_2 P25 nanopowder, commonly used as a benchmark in photocatalytic testing (Fig. 1).

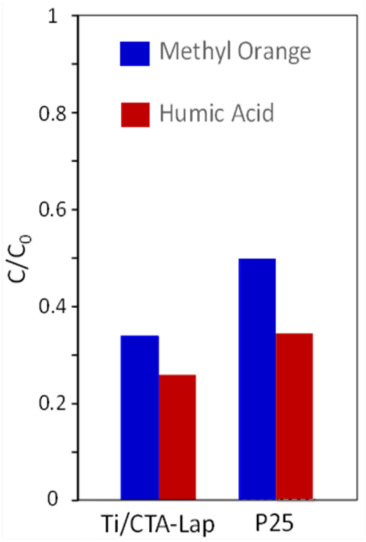


Fig. 1. Comparison of the photocatalytic efficiency of the best TiO_2/clay composite catalyst (TiO_2 supported on organo-Laponite) and the P25 reference in the destruction of methyl orange (5×10^{-5} M aqueous solution) and humic acid (40 mgL^{-1} aqueous solution) upon 5 h irradiation with a 365 nm 50 W LED lamp.

3. Coarse-grained modeling of carbohydrates

"Opus" research project NCN 2019/35/B/ST4/01149 [2020-2025]
(Project leader: Professor Wojciech Płaziński)

Conformational free energy calculations using the Martini 3 force field. Development of a model for rotating linkages and flexible rings in carbohydrates within Martini 3.

In the first part of the study, a computational protocol was developed and tested, which allows for the estimation of relative changes in free energy resulting from conformational changes in protein molecules induced by various factors (mutations of amino acid residues, variable pH values, solvent changes, etc.). The developed protocol is based on the coarse-grained (CG) Martini force field (version 3) and uses molecular dynamics (MD) simulations based on gradual changes in the λ parameter, which describes conformational changes between two predefined conformational states. The free energy change associated with such a transition is calculated using any method based on a formalism that utilizes λ (e.g., thermodynamic integration, Bennett acceptance ratio, or umbrella sampling). This method was tested on five different biomolecular systems, where conformational changes of proteins are induced by modifications in the conditions in which the protein molecule is located. The proposed approach is simple and applicable within the standard procedure used for conducting Martini-based CG MD simulations. It only requires knowledge of two (or more) conformational states defining the considered conformational transition and the ability to implement factors affecting this transition. The described procedure may be particularly useful when considering various factors acting on the same system, which may disrupt the dynamic equilibrium between specific, well-defined protein conformations. The results were published in the article: [10.1021/acs.jctc.3c01155].

In the second part of the study, the development of a model was addressed, enabling the inclusion of selected conformational degrees of freedom (rotation of glycosidic linkages along with side anti- conformers and distortions of pyranose rings) in the newly developed [10.1021/acs.jctc.2c00553] Martini 3 force field designed for carbohydrate simulations. An approach was developed that involves collecting reference data separately for all stable or metastable conformers and then combining these data sets with a weight corresponding to their relative populations under standard conditions (determined in atomistic simulations). Such data can be used, for example, to determine intramolecular interaction potentials with high accuracy, considering various secondary and tertiary energy minima that are typically neglected in the CG parameterization process. The developed method is currently in the implementation phase, enabling automatic, efficient, and flexible (with respect to a large number of possible systems) generation of CG force field parameters.

4. External stimuli-assisted Molecular Beam Epitaxy of functional layers and nanostructures

"Opus" research project NCN 2020/39/B/ST5/01838 [2021-2024]
(Project leader: Professor Józef Korecki)

Magnetite is an exceptional compound due to the combination of magnetic and transport properties that is rare for oxides. Its quasi-metallic conductivity at elevated temperatures abruptly drops by two orders of magnitude across the Verwey transition (VT) at around 125 K. In parallel, the high Curie temperature makes magnetite thin films interesting for spintronics, but their magnetic and electrical properties undergo significant modifications with decreasing thickness. It has been reported that antiphase boundaries (APBs), which strongly influence the magnetic properties, are also responsible for a significant reduction in electrical conductivity. Here, we postulate and prove the additional origin of this effect.

Our research was motivated by comparing *in situ* and *ex situ* conversion electron Mössbauer spectroscopy (CEMS) measurements on ultrathin magnetite films grown under UHV conditions. We found that these films oxidize toward maghemite when exposed to the atmosphere. For this reason, to reveal the true character of the thickness dependence of the resistivity in magnetite, the electrical measurements should be performed *in situ* under UHV. This was possible using special sample holders with electrical contacts to molybdenum pads predeposited on MgO(001).

Epitaxial magnetite Fe_3O_4(001) films with different thicknesses were grown on MgO(001) by reactive deposition of Fe under the O_2 partial pressure of $5*10^{-6}$ mbar on the substrates kept at 530 K. We measured a thickness and temperature dependence for a series of the Fe_3O_4(001) films in the thickness range from 2 nm to 50 nm. The thickness dependence was discussed based on classical models, and the contribution of APBs is estimated. For the available measurement temperatures (T_{min}= 100 K), we found VT for films as thin as 5 nm. The effect of oxygen adsorption and prolonged storage in the ambient atmosphere was also discussed.

We have also studied Morin transition (MT) in epitaxial hematite films grown by molecular beam epitaxy (MBE) on Pt(111). Films grown under typical conditions have not shown MT down to 110 K. Recently, a hematite film was grown in the presence of the external magnetic field using the newly developed technology of the magnetic-field-assisted MBE. Using CEMS, we found that this film exhibited a weak uniaxial anisotropy induced during the growth. Moreover, CEMS measurements at low temperatures revealed an onset of the Morin transition, which is absent for hematite films on Pt(111) without a magnetic field. The XMLD-PEEM imaging performed at SOLARIS synchrotron at room temperature confirmed an antiferromagnetic (AFM) domain structure expected for uniaxial anisotropy. Eventually, we performed low-temperature imaging at 70 K, which proved the spin-flop transition from the in-plane AFM domains to an out-of-plane configuration. Transition is incomplete, and the perpendicular spins coexist with the in-plane AFM phase.

5. New generation teranostatic nanocarriers for detection, diagnosis and neuroprotective treatment of ischemic stroke brain damage

"Opus" research project NCN 2020/39/B/NZ7/01913 [2021-2025]
(Project leader: Professor Piotr Warszyński)

Ischemic stroke is one of the major causes of death or long-term disabilities worldwide; thus, detection and treatment of ischemic-related damage, being still largely unresolved problems of contemporary medicine, require new technologies for diagnostics and therapeutics. Moreover, referring to the present pandemic, there is mounting evidence that patients affected by COVID-19 may develop clinically significant complications, including ischemic stroke. The main limitation of current ischemia/reperfusion injury treatment is an inefficient delivery of neuroprotective drugs to the affected part of the brain due to the blood-brain barrier (BBB) being permeable only by small, lipophilic molecules. Additionally, some neuroprotective drugs may influence the body, causing peripheral toxicity and numerous adverse reactions. The term "theranostic" describes the integration of therapeutic and diagnostic functions in one modality. The theranostic drug carriers can deliver their therapeutic cargo to the desired organ. Simultaneously, the delivery can be monitored by some imaging techniques for optimizing targeting and dosage. The diagnostic agents used for imaging are magnetic resonance imaging (MRI) or computed tomography contrast agents. Theranostic drug carriers combine the ability to load lipophilic drugs with high efficiency with their detectability with some imaging techniques. Moreover, they should transport the drug to the targeted place in the organism without its loss and release it in the area of action without imparting the therapeutic effect.

The main project objective, realized by the consortium of four research groups from Jerzy Haber Institute of Catalysis and Surface Chemistry of the Polish Academy of Sciences, Jerzy Maj Institute of Pharmacology PAS, Henryk Niewodniczański Institute of Nuclear Physics PAS and Collegium Medicum Jagiellonian University is to develop the new strategy of delivery of selected neuroprotectants by theranostic nanocarriers that can cross BBB without side effects on its normal function.

In 2024, we focused on the formation of theranostic nanocarriers with polymeric or nanoemulsion core and polyelectrolyte shell (core@shell nanocarriers) of selected neuroprotective drug cannabidiol (CBD). CBD-loaded nanocores (both types) were modified by sequential adsorption of polyelectrolytes with gadolinium-labeled poly-L-lysine (PLL-Gd) for MRI and poly glutamic acid (PGA). The size of prepared nanocarriers were ~150 nm. The effects of the developed nanoformulations on the viability of the human neuronal-like SH-SY5Y cell line were evaluated.

At the project's following stages, the neuroprotectants' effects in the theranostic formulations on the organotypic hippocampal slice culture exposed to oxygen-glucose deprivation (OGD) will be examined. Moreover, the impact of theranostic formulations on the harmful factors of OGD will be measured. In vivo experiments will determine the biodistribution of nanocarriers and the efficiency of transport to the brain. At the final stage of the project, the effect of the nanocarriers containing neuroprotectants will be tested in the middle cerebral artery occlusion (MCAO) animal ischemic stroke model.

6. Elaboration of fast and simple physicochemical method of detection of water pollution based on monitoring of dynamic properties of liquid/gas interface

"Opus" research project NCN 2021/43/B/ST8/00053 [2022-2025]
(Project leader: Professor Jan Zawała)

The project is aimed to elaborate the fundamentals of a simple physicochemical method for detecting surface-active water contaminants (SAC), based on monitoring dynamic changes in the interfacial liquid/gas surface properties. In practice, to determine the concentration of SAC in aqueous systems, parameters of the motion of single air bubbles floating in the liquid will be used. This approach is based on the fact that the liquid/gas surface properties in dynamic conditions are extremely sensitive to the presence of even trace amounts of organic substances in the solution (as low as ppm levels).

During the reporting period, work continued on constructing a prototype system designed for the automatic measurement of the velocity of a bubble floating in the liquid (aqueous solution). The prototype device was upgraded by developing a single-bubble generator based on pressure analysis in the system and creating a device casing using 3D printing technology. This casing was designed to accommodate a phototransistor matrix. Additionally, an electronic system was developed for measuring the current signal induced by the presence of a bubble, and an automatic data acquisition system was designed. Its operating principle involved fitting a Gaussian function to the collected experimental points and automatically reading the distance between maxima (see Fig. 1A). The developed software then returned the average bubble velocity at any chosen distance. During the reporting period, it was confirmed that the developed method has significant potential, as the obtained results were entirely consistent with measurements using the traditional method, i.e., recording bubble motion with a camera and analyzing the image (measuring the distance between the geometric centers of bubbles moving due to buoyant force using graphical analysis software). This effect is illustrated in Fig. 1B, where the average terminal bubble velocities in solutions of n-hexadecyltrimethyl-ammonium bromide (CTAB) obtained using both methods are compared. The next step will involve connecting more phototransistors and using such a matrix to measure so-called bubble velocity profiles. This will enable the development of appropriate calibration methods that can be used to identify the type and concentration of surface-active substances in water samples collected from natural waters and wastewater.

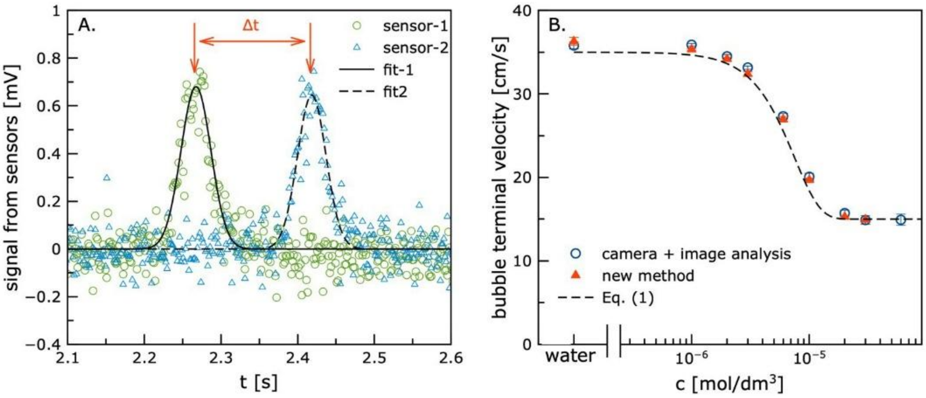


Fig. 1. Data illustrating (A) the signal obtained from phototransistors with a fitted Gaussian function, used to calculate the average bubble velocity in an aqueous solution, (B) a comparison of the average terminal bubble velocities measured using two methods in CTAB solutions of varying concentrations

7. Excited states under magnifying glass – adaptation of approaches based on density analysis for investigation of electronically excited molecular states

"Opus" research project NCN 2021/43/B/ST4/02969 [2022-2026]
(leader: UW, ICSC PAS partner: Associate professor Dorota Rutkowska-Żbik)

The aim of the project is to propose a systematic examination of the QCT applicability and especially its extension with new tools, devised specifically for excited states of various types, such as valence, charge-transfer, core, or Rydberg.

The porphyrin-based molecule (tetrakis(4-carboxyphenyl)porphyrin (TCPP)) and its double protonated form, H_2TCPP^{2+}, were selected for theoretical studies. The aim of the study was to explore how partial protonation of porphyrin affects the nature and properties of photoexcited states of TCPP. The studies comprised Density Functional Theory calculations of the ground S_0 state and the first excited triplet state T_1 of TCPP and H_2TCPP^{2+}. For structure optimizations and electronic structure calculations, we utilized the Gaussian 09 program. The dihedral angles of the porphyrin core in TCPP increased only slightly when transitioning from S_0 to T_1 geometry, consistent with previous experimental and computational findings. This observation contrasts with the structural changes observed upon protonation. We found strong ruffling of the tetrapyrrolic unit in the protonated S_0, which is even more pronounced in the protonated T_1 geometry. The ground-state findings align with experimental steady state UV/vis spectra, which showed a redshift of the Soret band due to the disruption of the π-system caused by ruffling. We proposed that the reduced triplet state lifetime observed upon excitation of protonated ground-state molecules is a result of the changed geometry.

From natural population analysis, we derived the charges of the pyrrolic nitrogen atoms. Here, a higher uniformity between the charges of the pyrrolic nitrogen atoms at T_1 was observed. Furthermore, electron density difference maps were derived for the ground state and the triplet state. These maps revealed increased electron density, and thus nucleophilicity, at the unprotonated nitrogen atoms in the porphyrin plane. We were able to explain the increased basicity of the first excited triplet state, as derived from transient absorption spectroscopy experiments. This finding is consistent with the presence of a free lone-pair orbital that binds the hydrogen atom upon protonation. The electron density decreased above and below the porphyrin plane and corresponds to the p-orbitals, which are not involved in the protonation.

8. Structure and Function of Protein Corona at the Nanoparticles Interface

"Opus" research project NCN 2021/41/B/ST5/02233 [2022-2026]
(Project leader: Professor Barbara Jachimska)

The main objective of the project entitled "Dendrimers as a platform for designing biologically active carriers" is to create a scientific basis for understanding the mechanism of protein adsorption on the surface of carriers based on PAMAM dendrimers and to develop a methodology for obtaining stable hybrid structures with appropriate functionality in a controlled manner, which can be used as drug carriers, materials for immunological tests or theranostic materials. The applications of dendrimers in targeted drug delivery systems are caused by the rapid development of nanopharmacology. The structure of a dendrimer potentially allows for two types of encapsulation of a therapeutic agent: on the multilayer dendrimer shell or inside the molecular structure. A dendrimer can play a dual role in delivering or releasing an active substance. Understanding the nature of the interaction between drugs and the carrier is essential for understanding this system's molecular mechanism of optimization and control. All nanocarriers interact with biological fluids after being introduced into a biological system. Thus, to develop selective drug delivery using carriers, it is necessary to understand both the phenomena associated with conformational changes and the competition of proteins for the carrier interface.

The project implementation includes three main research stages. The first stage of the research concerned the interaction of plasma proteins with the PAMAM dendrimer surface and the characteristics of the protein shell formed on the carrier surface (the so-called soft corona). At this stage, it was essential to determine the stability of the complexes formed and the changes in the native structure of proteins adsorbed on the interface. The second stage of the research included optimizing the interaction of PAMAM dendrimers with selected therapeutic substances by creating a stable bioactive dendrimer complex. This research stage is crucial and provides the basis for choosing systems for the final research stage on the effect of plasma proteins on bioactive dendrimer complexes. Only conformationally stable structures were selected for this phase of research. As part of the research conducted at the current stage, systems based on G4.0 PAMAM with 5-fluorouracil (G4.0-5FU) and G4.0 PAMAM with doxorubicin (G4.0-DOX) were optimized. Complementary research methods were used at each stage. Experimental studies were supplemented by molecular dynamics (MD) simulations. Computer simulations were performed for selected experimental systems with the most significant application potential (G4.0-5FU and G4.0-DOX) and confirmed the mechanism of drug immobilization in the carrier structure. In vitro biological studies of G4.0 PAMAM complexes with doxorubicin (DOX) and 5-fluorouracil (5-FU) showed their therapeutic potential. In cell models, the highest anticancer activity was noted against A375 malignant melanoma cells, emphasizing the possibility of using this complex in oncological therapy. For example, it was found that the preferred conditions for DOX binding to the dendrimer occur in an alkaline environment when the DOX molecule is deprotonated, which promotes its stabilization and controlled release in cancer cells. Fluorescence studies and drug release curves indicated the reversible nature of DOX binding to the nanocarrier, which is crucial for limiting systemic toxicity. Notably, the tested complexes showed a preferred intracellular localization (the fluorescent properties of DOX were used) compared to the free form of the drug, which increases their therapeutic efficacy and specificity towards cancer cells. Additionally, the complexes developed within the project were functionalized with plasma proteins to improve the efficiency of uptake by cancer cells. The results confirm that G4.0 PAMAM dendrimers may be a promising carrier platform for anticancer drugs such as doxorubicin or fluorouracil.

9. New generation of multi-charge surfactants of dedicated functionality

"Opus" research project NCN OPUS 2022/45/B/ST4/01184 [2023-2026]
(ICSC PAS partner: Professor Piotr Warszyński, project coordinated by prof. K.A. Wilk, Wrocław University of Science and Technology)

The main project objective is to develop the scientific background of a novel strategy to design and fabricate multicharge surfactants of dedicated functionality and evaluate their usefulness. Several designed groups of surfactants will be synthesized with various ionic dendronium-based headgroups. A specific focus of this project is based on the research hypothesis that it is essential to ascertain a link between the surfactant molecular architecture and the resulting structures - as the structural parameters and interfacial behavior of the multicharge surfactants both at interfaces and in solution - are essential for understanding their adsorption and aggregation properties and a variety of potential applications.

The interfacial behavior of aqueous solutions of newly synthesized 2-alkyl-N,N,N, N’,N’,N'-hexamethylpropan-1,3-ammonium dibromides with decyl, dodecyl, and tetradecyl alkyl chains was investigated both experimentally and theoretically. The results of the surface tension measurements were described using the modified surface quasi-two-dimensional electrolyte (mSTDE) model of ionic surfactant adsorption, which was supported by molecular dynamics simulations. Our contribution encompasses the design, synthesis, and characterization of a novel class of dicephalic-type cationic surfactants, branched on a methine motif, possessing two symmetric trimethylammonium groups, which constitute a double-head extension of the standard alkyltrimethylammonium salts of the single-head, single-tail structure. The convenient synthetic route and final purification steps allowed for the high-yield, high-purity production of the surfactants. Dicephalic-type surfactants demonstrated lower surface activity and higher critical micelle concentration (CMC) values when compared with their single head–single tail counterparts. That can be attributed primarily to the presence of strong electrostatic repulsive forces within the bulky, double-charge headgroups and significant counterion condensation. Furthermore, molecular dynamics simulations demonstrated a propensity for desorption of surfactants from the interface, even in diluted solutions, which constrained the attainable surface concentration and resulted in a lower reduction in surface tension. The mSTDE model of adsorption provided an excellent description of the experimental surface isotherms with a concise set of parameters. The model’s predictive power was demonstrated by the studies of the effect of inorganic salts on the surface activity of the investigated surfactants. Our unique approach enabled us to gain a theoretical explanation of the newly devised surfactants’ behavior at the water/air interface.

10. Mechanisms of cyclization reactions leading to biologically active compounds catalyzed by iron dependent enzymes

"Opus" research project NCN 2022/45/B/ST4/01411 [2023-2027]
(Project leader: Professor Tomasz Borowski)

The obtained results concern the structural and functional properties of iron-dependent enzymes, which play a key role in the cyclization of natural compounds. Cyclization often changes the properties of compounds and thus their activity. A classic example is the ring-opening reaction used by bacteria in beta-lactam antibiotics, leading to their deactivation. The research carried out in 2024 focused on the enzyme extradiol dioxygenase L-DOPA (DODA), which plays a key role in the biosynthesis of antioxidant pigments - betalains. This occurs by stereoselective cleavage of the bond between carbon atoms 4 and 5 of L-DOPA. The use of an improved protein purification protocol allowed for obtaining higher yields from one liter of medium. The thermal stability of the purified protein was checked using the FTS (fluorescence thermal shift) method, showing the highest stability in buffers with pH 7.5-8. Additionally, it was found that the addition of glycerol significantly improves protein stability. The spectrophotometric method of measuring enzyme kinetics was standardized and the optimal reaction conditions were established by selecting the MES buffer at pH 6.0 as optimal for the activity of the BvDODA enzyme. The Michaelis-Menten constant (KM) for L-DOPA was determined at 0.14 mM, and the reaction model was defined as substrate inhibition. The conducted studies allowed for precise determination of the enzyme kinetic parameters, which is crucial for further studies on the mechanisms of cyclization of biologically active compounds. Moreover, the obtained protein preparation allows for conducting experiments using the Mössbauer and stopped-flow methods, as well as measurement of kinetic parameters with respect to oxygen as a substrate using an oxygen electrode. Using the sitting drop method, DODA crystals bound to inactive ligand analogues: mimosine and 4-nitro-L-DOPA were obtained. Diffraction data were collected using the P13 beam of the DESY synchrotron (EMBL, Hamburg). The structures of the proteins bound to the ligands were solved using molecular replacement and then refined with the phenix.refine software.

The conducted mechanistic computational study aimed to discover the mechanism governing the stereoselectivity of DOPA with respect to other dioxygenases that catalyze the cleavage of the bond between carbon atoms 2 and 3 of L-DOPA. Molecular docking and molecular modeling were used to determine the possible orientations of L-DOPA during binding to the active site of the studied enzyme in selected alternative protonations of L-DOPA. Quantum-mechanical calculations allowed obtaining potential energy diagrams of intermediate products and transition states based on the assumed mechanism. Analysis of energy barriers allowed determining the optimal protonation state of L-DOPA and the predicted site of carbon-oxygen bond formation. The results are consistent with the nature of the reaction product obtained experimentally.

A comparison of sequences and structures of DODA enzymes available in the literature and their variants was performed. They were compared, specifying differences in amino acid residues found in analogous structures of individual DODA enzymes, as well as specifying differences within conserved amino acids. Appropriate statistics were created and the obtained data were compared in the context of differences in the activity of the enzymes studied. The literature review and subsequent bioinformatic studies allowed us to specify ten species of organisms producing DODA enzymes, whose further studies are relevant to the project's goals.

11. Biodegradable, biocompatible and interactive surfactants - as an ecologically safe alternative to synthetic compounds in the generation of foams and emulsions for cosmetic, medical and industrial applications

"Opus" research project NCN 2022/45/B/ST8/02058 [2023-2027]
(Project leader: Marcel Krzan PhD)

During the project, I want to solve the fundamental research problem concerning the influence of adsorption processes on the phase parameters of the dispersion system and develop new, safe foams/emulsions with excellent biocompatibility and biodegradability along with technologies for their production to reduce the negative impact of traditional chemical surfactants.

To achieve these goals, I need to explain the influence of molecular structure and dynamic adsorption effects of surfactants at different hierarchical levels in liquid functionalized foams and emulsions from the nano to the macroscopic scale. Therefore, I propose conducting a series of tests using safe model surface-active compounds that interact with "intelligent" and natural bioactive or therapeutic biopolymers or nanoparticles.

For this purpose, I study the properties of adsorption and foam/emulsion generation at all levels: from molecular interactions, where the parameters of the adsorption layer will be analyzed using various tensiometers, rheometers and spectrometers (including Sum Frequency Generation spectroscopy), through the rheology of thin foam layers and their stability (Thin Liquid Film Pressure Balanced Technique) to the study of the structure, stability and rheological properties of foam/emulsion.

During the project's second year, the properties of foams and emulsions stabilized with the biosurfactant saponin were studied, the adsorption properties of which were modulated by additives that were acceptors or donors of hydrogen bonds. Choline chloride, urea and glycerol were used as additives for foams. In the case of emulsions, the list of additives was extended to include betaine, thiourea and nicotinic acid.

As a result of the work, it was shown that both the adsorption parameters and the properties of the created dispersion systems (foams and emulsions) change drastically under the influence of additives (attached is a graph of changes in adsorption parameters for the tested systems with Sum Frequency Spectroscopy).

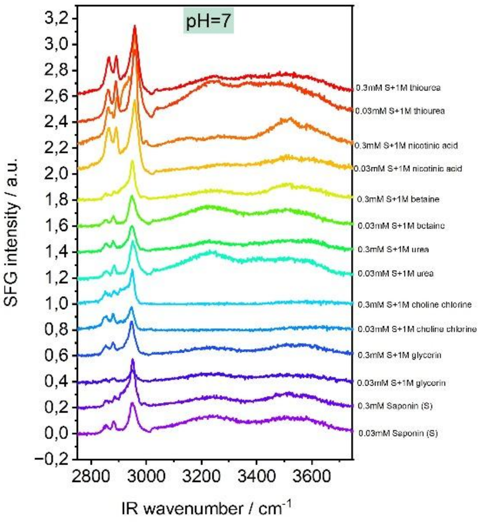


Fig. 1 Changes in SFG IR spectra of saponin under the influence of different additives (hydrogen bond acceptors and donors) at pH 7

12.From Single Molecules to Stress Granules – Understanding the Mechanisms of Phase Separation of Proteins linked to Amyotrophic Lateral Sclerosis

"Opus" research project NCN 2021/43/B/ST8/01900 [2023-2027]
(Project leader: Anna Bratek-Skicki DSc)

In 2024 a further set of experiments was performed to determine the essential characteristics of hnRNPA2 and TDP 43 in solution and their oligomerization under various pH and ionic strength conditions. By applying theoretical calculations and various experimental techniques, a comprehensive insight into the intrinsically disordered protein oligomerization mechanism was gained. Particularly, the focus was on developing an effective method for determining the degree of oligomerization of the hnRNPA2 Low Complexity Domain.

AFM investigations of the hnRNPA2 LCD molecule adsorption on mica under diffusion-controlled transport furnished reliable information about the stability of its solutions, particularly about the oligomerization degree (Fig.1). It was confirmed that the protein layer consisted of quasi-spherical oligomers with the size depending on the storage time. It was also shown that the oligomerization degree increased with the ionic strength of the electrolyte suggesting a significant role of the electrostatic interactions. The oligomerization mechanism of the protein was also effectively investigated by applying the adsorption at carrier particles where the monolayer formation time was considerably shorter than for planar substrates. Additionally, the precision of these measurements was larger, therefore reliable results could be acquired for nanomolar amounts of the protein. These experimental results were quantitatively interpreted in terms of the electrokinetic model that yielded valid information about the protein oligomerization degree and its surface coverage. This knowledge can be exploited to develop a facile method for quantifying the oligomerization kinetic of unstable protein solutions. One can also expect that our results enable to develop a procedure for preparing stable polymer particle conjugates with a controlled coverage of the protein.

13. Tungsten Aldehyde Oxidoreductase - a novel hydrogenase. Studies of reaction mechanism and potential biocatalytic applications

"Opus" research project NCN 2023/51/B/ST4/01224 [2024-2028]
(Project leader: Professor Maciej Szaleniec)

The project aims at investigating the recently discovered new enzymatic activity of tungsten aldehyde oxidoreductase (AOR), which turned out to be a new type of hydrogenase. AOR's hydrogenase activity expands its activity beyond catalytic oxidation of a wide range of aldehydes to carboxylic acid to oxidation of hydrogen which can be coupled to the reduction of carboxylic acids to aldehyde or NAD^+ to NADH. AOR is the enzyme originating from facultative anaerobic bacteria, *Aromatoleum aromaticum*. It contains tungsten cofactor (W-co) in its active site, where metal ion is coordinated by two pterin cofactors that are additionally connected by magnesium ion. The W-co is linked by a chain of iron-sulfur clusters with the subunit containing the FAD cofactor, which is responsible for the transfer of electrons to the NAD^+ coenzyme. Therefore, the project's ultimate goal is to understand a new catalytic phenomenon and develop methods for its application.

The research on the project encompassed preparatory and organizational steps, such as the description of the genetic manipulation protocols for *A. evansii* (the chapter is under revision in Methods in Enzymology). Furthermore, A. evansii cultures were conducted at the 2 L scale with plasmids coding for two AOR variants (wild type and mutant version with truncated oligomerization helix). The bacteria were collected, and new stock cultures were prepared after checking the activity of enzymes in the oxidation of benzaldehyde. The storage tests for the stability of the cell extract stored at -20°C under aerobic conditions were also conducted indicating swift inactivation of the enzyme.

As the project was funded in mid-2024 the main activities were concentrated along the organization of the research group and procurement of the necessary equipment. The PhD recruitment campaign was organized during the summer holidays which unfortunately did not yield candidates that were able to pass the entry exam to PhD School. However, a new recruitment procedure was reinitiated in November 2024 which will be concluded in January 2025. Furthermore, cooperation with AGH University of Technology was initiated which led to a joint MSc project and recruitment of an undergraduate student who will be involved in the project starting the next semester.

Two equipment purchases were conducted – the thermostat for the glovebox was purchased and installed into the system restoring the possibility of cooling samples under anaerobic conditions. Furthermore, two tenders for LC-MS equipment were carried out and their settlement is expected by the end of December. Finally, a Material Transfer Agreement was concluded with the University of Amsterdam which will enable the shipment of bacteria with enzymes for the cascades producing amines.

The results of the preliminary research for the project were presented at the ESAB Congress and the open innovation offer was presented at the Life Science Open Space event (both events took place by the end of November).

14. Biochemical system of glandular trichome microenvironment, its biocatalytic properties and potential in biotechnology

"Opus" research project NCN 2023/49/B/NZ1/02898 [2024-2028]
(ICSC PAS partner: Professor Maciej Szaleniec, project coordinated by Paweł Rodziewicz DSc, Institute of Plant Physiology PAS)

Plant glandular trichomes are epidermal plant outgrowths specialized in the production, secretion and storage of a vast array of secondary metabolites. Many of these compounds have potent biological activities and are used as fragrances, dyes, plant growth agents, fragrances, pesticides, and foremost pharmaceuticals, e.g., cannabinoids.

The project aim is to explore the glandular trichome microenvironment of cannabis and tomato species on several levels of cellular organization – metabolomics, proteomic, and to some extent transcriptomic. Based on previous studies we presume that glandular trichome exudates are a functional part of the trichome machinery, and form a very specific solution which supports enzymatic catalysis *ex vivo*. In the project, we plan to research biocatalytic properties of glandular trichomes, and on the basis of obtained results recreate the trichome microenvironment, develop microemulsion models capable of catalysis, and demonstrate biochemical reactions such as oxidative cyclization, which we have shown already in our previous studies, but in a very basic setup.

The project is conducted in cooperation between the Institute of Plant Physiology PAS (IPP PAS) and the Institute of Catalysis and Surface Chemistry PAS (ICSC PAS) within Joint Laboratory of Biotechnology and Enzyme Catalysis. The study conducted in 2024 in ICSC PAS focused on the characterization of three enzymes: THCAS (tetrahydrocannabinolic acid synthase), CBDAS (cannabidiolic acid synthase), and CBCAS (cannabichromenic acid synthase). These enzymes play a key role in the biosynthesis of cannabinoids and exhibit high substrate specificity. The coding sequences for these enzymes were identified and retrieved from the UniProt and GenBank databases.

The genes encoding the enzymes were synthesized by an external biotechnology company and cloned into the pPICZalpha vector, which enables protein secretion in the *Pichia pastoris* expression system. The vectors were propagated initially in *Escherichia coli* cells and subsequently in *Pichia pastoris*.

At this stage, the research concluded with the successful transformation of the vectors containing the genes into the yeast host. The next steps will involve conducting a mutant screening to optimize the expression and activity of the studied enzymes.

15.Exploring metal ion cooperation in binuclear transition metal sites

“Opus LAP” research project NCN 2020/39/I/ST4/02559 [2021-2024]
(Project leader: Associate Professor Dorota Rutkowska-Żbik)

In the current project we aim at the exploration of the reactivity of the binuclear divalent transition metal ion (TMI) sites (TMI = Fe, Co, Mn, Ni) stabilized into the zeolitic matrices as models of the binuclear sites in enzymes. Last year studies were focused on 1) determination of reactivity of the model systems in which transition metal ions form a distant bi-nuclear site, for the activation of N_2O species and formation of the reactive oxygen species responsible for selective oxidation in model systems (bi-nuclear TMI sites located in zeolites are able to activate N-O bond and form a oxygen species on one of the metal sites); 2) establishing the catalytic tests of the zeolite-based materials in which transition metal ion sites were introduced either as single sites or as binuclear sites in the oxidative dehydrogenation of propane.

1) The process of N_2O activation by two distant cooperating TMI ions was studied using the model consisting of two metalloporphyrin species which were placed in parallel one to another at a varying distance (6,0 – 4,0 Å). The nitric(I) oxide molecule was placed in between the two TMI ions in parallel to the axis connecting two metal centers. The hydrogen atoms of both porphyrins were kept frozen, while the rest of the molecules were free to relax. The calculations were done within Density Functional Theory using Turbomole v6.3. def2-TZVP basis set was used with PBE functional for Ni, BP for Co and Mn, and B3LYP for Fe. Resolution of Identity (RI) approach, known also as density fitting, was applied for computing the electronic Coulomb interactions. The conducted studies did not show the ability of diatomic systems to break the N-O bond in the nitrogen(I) oxydie molecule for all tested ions (N_2O is not activated, the N-O bond practically does not change its length, and the molecule itself is arranged parallel to the Por planes), with the exception of $[Mn(Por)]_2$ (N-O breaking and formation of the Mn(IV)=O group are observed when the Mn-Mn distance is 4 – 4.5 Å.

2) The catalytic reaction (oxidative dehydrogenation of propane) was run in a fixed-bed gas flow reactor with a gas chromatograph to detect subtracts and reaction products at a temperature range from 200-500 °C, with varying contact times. The activity of the studied systems in the low-temperature oxidative dehydrogenation of light alkanes was investigated. Metallozeolites containing bimetallic centers (observed selectivity of Mn-FER and Fe-FER to alkenes ~100% at 200 ºC) were compared with those in which, due to their structure, transition metal ion pairs do not occur (active only at temperatures >350 ºC).

16.Preventive Conservation Strategies for Poly(vinyl chloride) Objects

“Opus LAP” research project NCN 2020/39/I/HS2/00911 [2022-2025]
(Project leader: Professor Łukasz Bratasz,)

This project aims to develop a preventive conservation strategy to determine the most economically and ecologically convenient conditions for storing heritage objects made of poly(vinyl chloride) – PVC. The research conducted within this project focuses on following the degradation process at temperatures not exceeding 80 ^{o}C by observing structural (chemical) changes occurring in the PVC matrix and correlating them with changes in the material's mechanical properties.

In the third year of the project, the focus was on the migration of orthophthalate plasticisers which are most frequently identified in museum collections containing PVC objects. The predominance of the inner diffusion or surface emission (evaporation), which determines the rate of the overall plasticizer migration process, was crucial for understanding the potential risk of orthophthalates emission and mechanical stress development. According to this concept, general correlations were proposed for the activation energy of diffusion and evaporation of various orthophthalates depending on their molar mass. The obtained relationships were created with the support of molecular dynamics simulations performed in cooperation with Prof. Tomasz Pańczyk from IKiFP PAN and solid-state NMR diffusometry performed by a team from the UAM NanoBioMedical Center in Poznań. These studies indicated that for the majority of PVC objects, the risk of accelerated migration after gentle removal of the surface layer of the plasticizer is low, and therefore cleaning the surface would reduce dust deposition and limit air pollution from objects emitting orthophthalates in the museum environment.

Considering the need for quick and simple solutions supporting decision-making regarding collection care, a step-by-step protocol consisting of non-destructive qualitative and quantitative analysis of plasticizers in objects made of plasticised PVC (infrared or Raman spectroscopy), determination of the physical state of the objects and rate-determining process of the migration was proposed.

The construction of a mechanical model in COMSOL Multiphysics® software for a representative inflatable object made of PVC foil with a thickness of 0.2 to 2 mm was completed using the material parameters determined in static and dynamic mechanical tests. Based on the obtained model, the maximum stress level - 5 MPa - generated in deflated objects during their storage was determined. Additionally, creep experiments were carried out to determine the deformation of the material under the influence of constant stress acting on the stored objects for an extended time. The obtained results indicated that a constant stress of 5 MPa causes approx. 10 times smaller deformation compared to the yield point, i.e. irreversible deformation. This means that the risk of mechanical damage (permanent deformation) in the case of inflatable objects made of thin PVC foil is negligible in practice.

The results obtained during the third year of the project were presented at national and international conferences (12 presentations). They were the subject of five publications (one published, and four under review). The project's main outcome will be guidelines on preventive conservation for artworks made of or containing elements of plasticised PVC.

"Sonatina" Research Projects of the National Science Centre

1. Multifunctional chitosan-based composite coatings for biodegradable Mg alloys

"Sonatina" research project NCN 2021/40/C/ST5/00266 [2021-2025]
(Project leader: Dzmitry Kharytonau PhD)

Magnesium alloys are extensively examined for being used as temporary biodegradable medical materials. Despite many advantages, such as biocompatibility and non-toxicity of Mg alloys and products of their degradation, they have several drawbacks. The main drawback of such materials is their rapid degradation in aqueous conditions. For this reason, new surface modification techniques allowing control of the degradation profile of Mg alloys should be developed. Chitosan is a natural polymer, which is promising for biomedical applications due to its nontoxicity, biocompatibility, biodegradability, film-forming ability, mechanical properties, and low cost.

The overall goal of the project is to develop a new type of multifunctional chitosan-based coatings on the surface of magnesium alloys with enhanced corrosion resistance and antibacterial functionality.

In the reporting period, the third and fourth specific objectives of the project have been examined:

Task 3. Analysis of mechanical properties of coatings.

In terms of Task 3, the project leader performed a 6-month international internship under the supervision of Prof. Nils Almqvist in the group Experimental Physics, Division of Materials Science, Department of Engineering Sciences and Mathematics, Luleå University of Technology (Sweden). In terms of this internship, the nanomechanical properties of chitosan films selected in tasks 1 and 2 were investigated by classical semi-contact atomic force microscopy (AFM) and intermodulation atomic force microscopy (ImAFM). The topography, nanomechanical response, and wear resistance of the coatings were investigated.

Task 4. Evaluation of corrosion performance and antibacterial activity of the chitosan and chitosan-based coatings. In terms of this task, selected chitosan and chitosan-based coatings deposited on the surface of Mg alloys and coordinated with phosphate and molybdate ions are now being examined for their short- and long-term corrosion behavior in Hank's solution.

Additionally, an in-depth examination of the corrosion properties of AZ31 and WE43 alloys in Hank's solution containing the molybdate inhibitor was performed. A multisine impedance-based tool for monitoring non-stationary electrochemical processes was proposed to examine the corrosion mechanisms of these alloys and inhibition performance of the inhibitor.

2. Manufacture of a new generation three-dimensional "drug-on-chip" materials with controlled release of active agents dedicated to personalised medicine

"Sonatina" research project NCN 2024/52/C/ST8/00148 [2024-2027]
(Project leader: Wiktoria Tomal PhD Eng.)

The use of classical drug delivery methods causes numerous problems and limitations related to low drug activity at the target location or negative effects on healthy areas of the body. To address these challenges, scientists have been developing drug carriers in the form of nanoparticles or nanocapsules, which have revolutionised the approach to pharmaceutical therapy. However, the increasing demand for more precise and controllable drug delivery systems has made the use of drug nanocarriers itself no longer sufficient, for example, for the delivery of immunosuppressive drugs. In addition, a number of additional requirements such as precise control of the drug release profile in the body, improved delivery of hydrophobic drugs, targeted drug delivery or prolonged drug release causes a constant search for new solutions that meet the listed requirements.

Considering the possibilities offered by the combination of nanotechnology and modern additive technologies, providing increasingly precise solutions enabling high personalisation of pharmacological treatment, a research plan has been proposed, allowing the synthesis and modification of polyelectrolyte drug nanocarriers, their implementation in photosensitive resins for 3D-VAT printing, and consequently manufacturing drug-on-chip materials with defined geometry, allowing controlled release of active substances. Therefore, the main aim of the project is to develop methods for manufacturing next-generation, three-dimensional "drug-on-chip" materials with controlled release of active substances, dedicated to personalised medicine.

Five research tasks are planned for the implementation of this project:

TASK 1:	Synthesis of polyelectrolyte drug nanocarriers;
TASK 2:	Determination of basic parameters of the obtained polyelectrolyte nanocapsules;
TASK 3:	Development of photosensitive nano-resin for 3D printing containing polyelectrolyte nanocarriers;
TASK 4:	3D printing of hydrogel materials with defined geometries containing polyelectrolyte drug nanocapsules using light-based printing technology;
TASK 5:	Analysis of the properties of the obtained drug-on-chip materials.

The proposed research project is a response to an identified research gap that is the lack of solutions to obtain hydrogel materials containing polymer nanocarriers in the form of polyelectrolyte nanocapsules using the 3D-VAT printing technique. In 2024, a preliminary study was conducted involving the manufacture of a 3D material containing nanocapsules with an active substance encapsulated inside. For this purpose, layer-by-layer synthesis of Rhodamine B functionalised nanocapsules was first undertaken. As a result of the LbL synthesis using poly(allylamine hydrochloride) (PAH) and poly(sodium 4-styrene sulfonate) (PSS) polyelectrolytes, 3-layer positively charged polyelectrolyte nanocapsules with an average size of 70 nm and zeta potential of 44 mV were obtained, which formed a stable dispersion in 0.015M NaCl solution. Subsequently, based on the experiments, the following base resin was selected: polyethylene glycol diacrylate (PEGDA, MW 575) with a TPO-Li photoinitiator (1 wt.%). This system is characterised by a short induction time (<1 s) and a high monomer conversion rate (~100%), which are desirable properties for the use of such resin in 3D printing processes. In the next step, the preparation of light-sensitive resins for 3D-VAT printing containing previously synthesized nanocapsules along with the above resin was carried out, and 3D printing of four designed models was implemented: 3 volumetric models with different geometries and one cuboid model, which was used for microscopic analysis.

3. Deciphering the unknown - structure and function of Nif3 protein

"Sonatina" research project NCN 2024/52/C/NZ1/00271 [2024-2027]
(Project leader: Elżbieta Wątor PhD Eng.)

The Nif3 protein has been identified as one of the most evolutionarily conserved yet least understood proteins among eukaryotes. Despite numerous studies on its prokaryotic homologs, the function of the eukaryotic Nif3—particularly human Nif3L1—remains unclear. There is evidence suggesting that this protein may be involved in various biological processes, such as DNA repair, methylation, iron homeostasis, cellular stress response, and may be linked to diseases including cancer, osteoporosis, and neurological disorders. However, current data are non-specific and insufficient to draw definitive conclusions.

The aim of this project is to fill this knowledge gap by employing an integrated approach involving structural biology, cell biology, proteomics, and transcriptomics. The central hypothesis is that Nif3L1 may be involved in polyamine metabolism, including the hypusination process of the translation factor eIF5A. The project combines targeted, hypothesis-driven studies with exploratory research designed to uncover novel functions of the protein.

The project officially started at the beginning of September 2024, but actual implementation began on October 15, 2024, with the hiring of the project leader. Due to the short duration of the project in 2024 (2.5 months), activities primarily focused on setting up the experimental infrastructure and initiating preliminary work.

The following actions have been carried out so far:

- **Purchase of equipment and reagents** – Orders were completed for essential computer equipment and laboratory reagents needed for experimental work.
- **Design of expression constructs** – Using bioinformatics methods, constructs were designed for the production of recombinant proteins in a bacterial system.
- **Optimization of protein production and purification** – Initial expression and purification tests were performed for proteins to be used in subsequent structural and functional studies.
- **Optimization of Nif3 protein crystallization** – Preliminary crystallization experiments were conducted using Nif3 protein from *Schizosaccharomyces pombe*. The resulting samples will be used in further analyses, including fragment screening, during an international research stay in Lund, Sweden.
- **Literature review** – An in-depth analysis of existing scientific literature on the Nif3 protein and its potential functions was conducted, which helped refine the direction of future research.

"Preludium Bis" Research Projects of the National Science Centre

1. Impact of metal and metal oxide nanoparticle functionalization by low-molar mass polyphenols on their activity in protein fibrillation processes

"Preludium Bis" research project NCN 2022/47/O/ST5/1858 [2023-2027]
(Project leader: Assistant Professor Magdalena Oćwieja)

Available literature data indicate that many types of metal and metal oxide nanoparticles play a key role in protein fibrillization processes, which are strongly associated with neurodegenerative diseases. Recent literature reports suggest that the activity of nanoparticles in fibrillization processes is closely related to their physicochemical properties. It appears that the dominant influence is exerted by the size of the nanoparticles and their surface properties resulting from the chemical structure of the stabilizing layer. The proposed hypothesis assumes that modifying the surface of nanoparticles with low-molecular-weight substances that have anti-fibrillogenic properties will result in systems that will inhibit protein fibrillization more effectively than free nanoparticles and selected inhibitors.

During the reported period, the work included: 1) the development of synthesis methods for titanium dioxide (TiO_2NPs), zinc oxide (ZnO), and iron oxide (Fe_3O_4) nanoparticles with similar morphology and size distribution, and 2) the development of efficient methods for surface functionalization of the aforementioned nanoparticles using gallic acid (GA) and rosmarinic acid (RA). Additionally, the aim of the planned studies was to carry out physicochemical characterization of both unmodified and polyphenol-functionalized nanoparticles.

Metal oxide nanoparticles were synthesized using chemical methods that combine hydrolysis and condensation processes (sol-gel method), precipitation and co-precipitation methods, as well as calcination. Parameters such as reagent concentrations, pH, ionic strength, temperature, mixing speed, and calcination conditions were optimized. As a result of the work carried out, quasi-spherical metal oxide nanoparticles with sizes ranging from 5 to 100 nm were obtained. Furthermore, imaging of the nanoparticles using transmission electron microscopy (TEM) provided information about the size distribution, average size, and polydispersity of the obtained systems. For each type of nanoparticle, studies were conducted using X-ray photoelectron spectroscopy (XPS), X-ray powder diffraction (XRD), and thermogravimetric analysis (TGA). The stability ranges of nanoparticles dispersed in aqueous electrolyte solutions with controlled pH and ionic strength were determined using dynamic light scattering (DLS). Additionally, electrokinetic properties of the nanoparticles (including electrophoretic mobilities, zeta potentials, and isoelectric points) were determined through measurements using electrophoretic light scattering (ELS).

The functionalization of metal oxide nanoparticles with gallic acid and rosmarinic acid was carried out based on: 1) electrostatic adsorption processes of polyphenols onto the surfaces of metal and metal oxide nanoparticles, and 2) a chemical reaction (esterification) between the polyphenols and hydroxyl groups on the surfaces of the metal oxide nanoparticles. Characterization of the nanoparticles using attenuated total reflection infrared spectroscopy (ATR-IR) showed the effectiveness of the functionalization process and the successful modification of metal oxide nanoparticles with gallic acid or rosmarinic acid on their surfaces. Electrokinetic studies revealed that the functionalized nanoparticles, unlike the unmodified ones, did not have isoelectric points and exhibited a negative zeta potential across the entire pH range (3-11), with the zeta potential value depending on the ionic strength. Based on the recorded XRD diffractograms, it was shown that the functionalization did not affect the crystalline structure of the oxides or the average size of the nanocrystallites.

2. Fine-tuning of polyelectrolyte complexes properties via ternary composition

"Preludium Bis" research project NCN 2023/50/O/ST5/00249 [2024-2028]
(Project leader: Assistant Professor Piotr Batys)

Simulation files were prepared for calculations of ternary polyelectrolyte systems using the molecular dynamics method. Poly(diallyldimethylammonium chloride (PDADMA) was selected as a model positively charged polyelectrolyte. On the other hand, the model negatively charged polyelectrolytes were poly(acrylic acid) (PAA) and poly(sodium 4-styrenesulfonate) (PSS). Input files for the $PDADMA_1$-PSS_{1-x}-PAA_x mixtures were prepared for x = 0, 0.2, 0.4, 0.5, 0.6, 0.8, 1. Moreover, each of the above systems was prepared in three different NaCl concentrations, i.e. 0M, 0.5M, 1.0M. All systems were equilibrated and simulations are currently being running on a supercomputer. An example initial configuration is shown in Fig. 1.

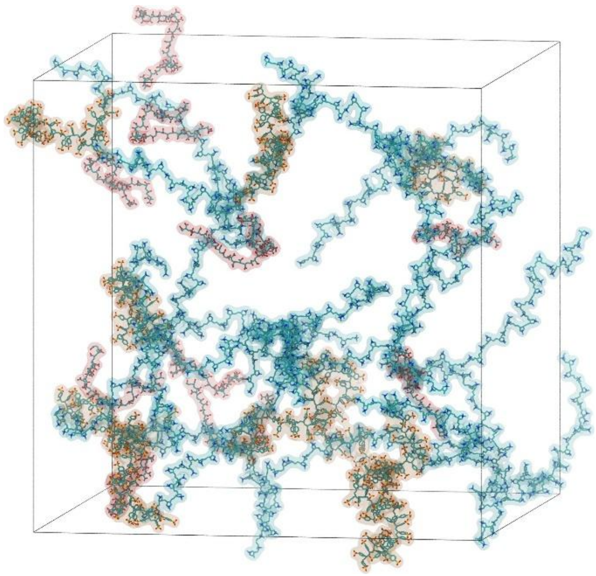


Figure 1. Initial configuration for the PDADMA1-PSS0.5-PAA-0.5 system. PDADMA, PSS, and PAA molecules are highlighted in blue, orange, and red, respectively

"Preludium" Research Projects of the National Science Centre

1. The analysis of the influence of selected factors on changes in α-synuclein structure

“Preludium” research project NCN 2020/37/N/ST4/02132 [2021-2024]
(Project leader: Paulina Komorek MSc, supervisor: Professor Barbara Jachimska)

The research planned as part of the project is a key step toward controlling the conformation of protein molecules and the structure of protein films on the adsorption surface. This is important for explaining the processes leading to the formation of abnormal protein structures characteristic of the development of neurodegenerative diseases, such as Parkinson's disease. Disorders of the musculoskeletal system mainly characterize Parkinson's disease and are primarily manifested by slow movement, tremors, muscle stiffness, and loss of coordination. A characteristic feature of the disease is the occurrence of Lewy bodies - abnormal aggregation of proteins that develops inside nerve cells. Studies of the composition of Levy bodies have shown that they mainly contain the protein α-synuclein, which occurs in incorrectly folded forms and can cause the death of neurons. Controlling the process of protein conformational changes is a key step in developing innovative therapies, developing strategies to prevent this type of disease, and designing modern diagnostic tools that enable rapid and precise detection of diseases at their early stages.

Using several advanced experimental methods, the transformation of monomeric proteins into protein aggregates in the solution phase and at interfaces was monitored in situ, mimicking protein aggregation in cells and tissues. The use of a coordinated methodological approach, including the use of quartz crystal microbalance (QCM-D), surface plasmon resonance (MP-SPR) and infrared spectroscopy (IR), allows for understanding the biochemical process at the submolecular level. Circular dichroism (CD) and infrared spectroscopy (IR) spectra confirmed that the interaction of the protein with the gold surface causes changes in its conformation, direction, and intensity of structural changes, which depend on the pH of the environment in which the adsorption was carried out. Complementary experiments using MP-SPR and QCM-D showed that the adsorption conditions and molecular structure have a more significant effect on the hydration of the resulting films than the degree of their surface coverage. The conducted studies aimed to determine the influence of molecular interactions on the stability of an internally disordered protein at the phase boundary. It was essential to identify the mechanism of changes occurring in the protein structure, leading to the formation of toxic aggregates and amyloid fibers in the final stage.

2. Development of evidence-based environmental specifications for short religious, cultural and commercial events in historic buildings

“Preludium” research project NCN 2020/37/N/HS2/01727 [2021-2023]
(Project leader: Magdalena Soboń MSc. Eng., supervisor: Professor Łukasz Bratasz)

Work in the project regarding the development of rational, evidence-based guidelines for climate control in historic interiors, which will allow curators and owners of similar buildings to make decisions to protect the cultural heritage remaining in their custody, mainly constituted by the massive wooden objects, i.e., sculptures and decorative elements was finalized.

The results of two experiments conducted using the acoustic emission method were analyzed, which were to serve to validate a two-dimensional model of a massive wooden element. The first experiment consisted in subjecting notched lime wood discs to a step RH change in a climatic chamber. The second experiment was based on monitoring the state of preservation of the sculpture of St. Sebastian exhibited in a display case at a temporary exhibition in the Wawel Royal Castle. As a result of the analysis, the use of experimental results for model validation was abandoned. The conditions of the first experiment differed from the microclimatic conditions representative of historical interiors (in particular RH in the range of 20-80%), while in the second experiment, due to logistical reasons related to the construction of the exhibition, the interval between the transfer of the sculpture from the Treasury of St. Mary's Basilica and the installation of the measurement system turned out to be too long to register a significant signal related to the change in RH between institutions.

The numerical model was used to analyze the impact of the following scenarios of microclimate fluctuations in St. Mary's Basilica (BM) in Krakow:

a) Heating the church interior in winter to a specified constant temperature level when the temperature drops below this value;
b) Heating the church interior to a thermal comfort temperature (T=18°C) for short-term religious, commercial, etc. events.
c) Moving the sculpture from the church interior to a conservation studio with a maintained microclimate considered optimal for works of art (T=21°C, RH=50%).

The analysis of each of the scenarios a)-c) consisted of calculating the energy release rate, G, as a function of the duration of the microclimate change and comparing it with the critical energy release rate, G_{Ic}, which is the threshold value for crack growth. A wide range of initial crack depths was considered.

It was determined that:

a) The highest temperature to which the interior of the BM can be heated in the winter without increasing the risk of damage to the altar sculptures is T=11°C.
b) Heating the BM to a thermal comfort temperature for short-term events, causing a drop to RH=20%, can last up to 12 hours without causing the risk of cracking the sculptures.
c) Moving the sculpture from the church to a conservation studio with a maintained climate considered optimal for works of art (T=21°C, RH=50%) for the duration of conservation should take place after a conscious risk analysis, because with a long-term change in conditions (9 months), a change even from RH=59% to 53% can cause an increase in the depth of cracks.

The project ended with the publication of guidelines for climate control in historic buildings and churches in the research paper in Heritage Science: Soboń and Bratasz, Risk of fracture in massive cultural objects made of lime wood: a case study of Veit Stoss’ altarpiece, Heritage Science (2024) 12:214

3. Synergistic foaming systems based on surface-modified magnetic nanoparticles and biodegradable amino acid surfactants in the controlled destabilization of real foams in a magnetic field

“Preludium” research project NCN 2022/45/N/ST8/02307 [2023-2025]
(Project leader: Mariusz Borkowski MSc., supervisor: Assistant Professor Jan Zawała)

As part of the project, foaming and foam-stabilizing properties were tested for selected systems composed of magneto-responsive nanomaterials and the amino acid-based surfactant C12-ARG. The obtained results show a strong correlation with previously acquired data on surface effects, as observed through surface tension measurements, SFG spectroscopy, and foam film stability studies. The data support the hypothesis that synergistic foam stabilization is achieved in systems with oppositely charged components. In the next stage of the research, the foaming properties of the pure surfactant and its mixtures with nanomaterials were examined at selected pH values using dynamic foam analysis. The experiments demonstrated that the addition of particles bearing a charge opposite to that of the surfactant at a given pH leads to increased foamability and enhanced foam stability under specific conditions. In the subsequent phase, these systems will be tested under the influence of a magnetic field using a newly developed measurement setup dedicated to analyzing foam-stabilizing properties in magnetic conditions.

"Beethoven Life" Research Projects of the National Science Centre

1. Structure and function of fumarate-adding glycyl radical enzymes: biochemistry, modeling and application

"Beethoven Life" research project NCN 2018/31/F/NZ1/01856 [2020-2023]
(Polish leader: Professor Maciej Szaleniec, German leader: Professor Johann Heider)

The project aim is the investigation of the biochemistry and reaction mechanisms of glycyl radical enzymes which catalyze the addition of fumarate (fumarate-adding enzymes, FAE). The prototype representative enzyme of the FAE class is benzylsuccinate synthase (BSS) from *Thaurea aromatica,* which catalyses radical addition of toluene to fumarate yielding (*R*)-bezylsuccinate.

The project was completed in the first quarter of 2024. In the last months of its implementation, we concentrated on summarizing the measurements and calculations and preparing publications.

In particular, kinetic equations describing the enzymatic reaction were derived, which allowed for a comparison of the experiment with QM:MM calculations that were carried out in previous years of the project. It was shown that the microkinetic model based on calculations correctly predicts the enantioselectivity of the reaction - the reaction rate leading to the product in the *R* configuration is 5766 times greater than that leading to *S*-benzylsuccinate. A similar analysis allowed for the correct prediction of the kinetic isotope effect (the predicted KIE value was 2.7 compared to the experimental value - 2.1).

Three mechanisms of isotope exchange in D_2O were also derived and described, and based on the kinetic analysis, the mechanisms leading to the formation of d^1-benzylsuccinate (Fig. 1A) and d^2-benzylsuccinate (Fig. 1B) were indicated. Kinetic analyses were also based on the barrier values obtained from QM:MM calculations and microkinetic analysis. Based on this approach, it was also possible to estimate the rate of isotope exchange H/D on cysteine in the active site (using the ratio of product concentrations observed in the experiment as a reference). The results of these analyses are described in a paper that has been deposited on the bioRxiv server.

Finally, the methodology for the genetic transformation of *Aromatoleum evansii* used in the production of recombinant BSS was described in a methodological chapter of the Methods in Enzymology series. The text of the chapter is currently under review.

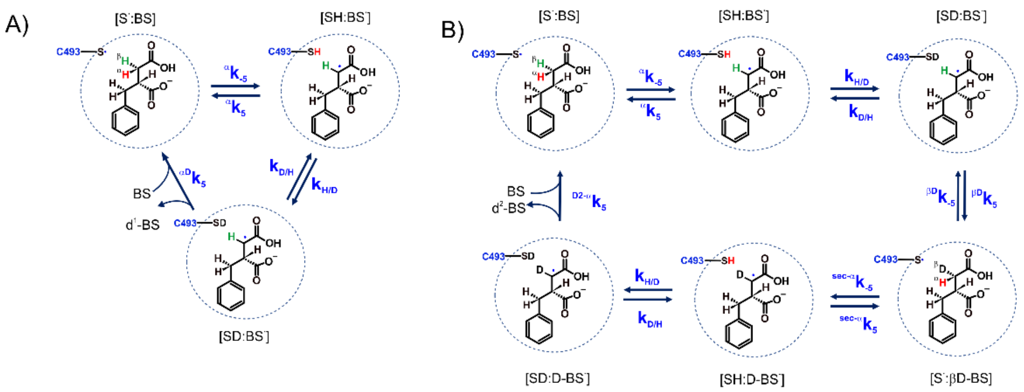


Fig. 1. Mechanisms of H/D exchange leading to A) d1-benzylsuccinate, B) d2-benzylsuccinate

"Solar-Driven Chemistry" Research Projects of the National Science Centre

1. SolarMethaChem: Solar light-driven photochemical processes for methane chemical conversion to valuable product

„Solar-Driven Chemistry" research project NCN nr 2019/01/Y/ST4/00024 [2020-2024]
(Project leader: Associate Professor Dorota Rutkowska-Żbik)

The aim of the research conducted within this project was to propose models of photocatalytic systems tested by the project's foreign partners and to investigate their electronic structure as well as to determine the mechanism of methane to ethane coupling in selected systems.

Theoretical studies considered the possibility of activating the C-H bond in methane under the influence of the so-called reactive oxygen species (ROS) – ·OH, ·OOH, $^1O_2$, which are formed in the presence of photocatalysts, from surface molecules present in the reaction medium (mainly O_2 and H_2O). The conducted studies (MP2/6-31g*) indicate the importance of this path of methane activation under photocatalytic conditions: the calculated energy barrier for the C-H bond cleavage in methane is 15.4 kcal/mol for the reaction with ·OH and 19.8 kcal/mol for the reaction with $^1O_2$. The reaction with ·OOH is not favorable (activation barrier 73.8 kcal/mol). The concentration of ROS and their relatively short lifetimes mean that the reaction paths with ROS are only one of the possibilities, not excluding reactions occurring on the photocatalyst surface.

The methane coupling step was studied in Ag-HPA-TiO_2 and Pd-HPA-TiO_2 systems. Experiments conducted in parallel with theoretical calculations indicated that in the above systems, methyl radicals are formed largely as a result of a gas-phase reaction, in the reaction between methane and hydroxyl radicals formed from water (as a reactant), as a result of a photocatalytic reaction. The calculations performed showed that $\cdot CH_3$ binds to the surface of photocatalysts. Pd nanoparticles and isolated Pd atoms bind $\cdot CH_3$ more strongly than silver ions, while the energy required to form a C-C bond is lower for the Ag-HPA-TiO_2 catalyst (8.8 kcal/mol) than for Pd-HPA-TiO_2 (36.6 kcal/mol) or Pd-TiO_2 (29 kcal/mol).

"GRIEG" Research Projects of the National Science Centre

1. Craquelure – Model of paintings with craquelure patterns for evidence-based environmental control in museums

"GRIEG" research project NCN 2019/34/H/HS2/00581 [2020-2024]
(Project leader: Professor Łukasz Bratasz)

The 2024 was the last year of implementing the GRIEG Craquelure project. The research team concentrated on applying the developed 3D physical models of canvas and panel paintings with developed craquelure patterns to determine safe ranges of relative humidity variations.

In panel paintings the formation of a new crack in layered materials is due to stress transfer from the substrate to the cracked layer, the tension in the middle between two cracks was modelled as a function of the magnitude of loading induced by moisture-induced dimensional changes of the layers building the painting. For paintings on panels, ratios of distances between cracks in the tangential and longitudinal directions of a wood panel to the layer thickness were estimated. The amplitude of relative humidity variation causing the development of new cracks was determined. It depends on panel thickness, cut of the wood, type of wood thickness of ground and paint layers, and distance between cracks. It was shown that for the normalised distance between cracks ca.3 the new cracks cannot be formed independently even for very large relative humidity variations.

The mechanism of crack formation in canvas paintings is different. The wooden stretcher sets a constraint on the canvas and ground layer response to relative humidity variations. The risk of crack formation is very low for both very stiff and very soft stretchers. The medium stiff stretchers are the worst case, leading to stress concentration in the corners. It was shown that the risk of paint layer cracking depends mainly on the relative humidity level at which the canvas was stretched. No cracking was observed if the canvas was stretched at 50% even for a relative humidity drop as large as 40%. If the canvas was stretched at 80%, the drop by 32% was causing cracking. The modelling has shown that similarly to panel paintings, cracks in canvas paintings significantly reduce the risk of further cracking. The critical ratio of the distance between cracks in the corners normalized to the thickness of the ground layer corresponding to the crack saturation depends on ground layer thickness and was estimated to 25-50 for ground layer thickness between 150 and 300 microns.

The findings of the project were implemented on the HERIe preventive conservation platform (herie.pl). The models of mechanical risk in panel painting were refined by adding an option for the user to select the distance between cracks. Obviously, for small distances, it reduces the risk of further cracking induced by relative humidity variations.

The achievements of the project were presented during the organised international symposium “Preventive conservation of paintings in heritage institutions” as well as at 4 training workshops “HERIe – preventive conservation platform” organised at Royal Wawel Castle, Warsaw University and Nicolaus Copernicus University.

2. Theranostic nanocarriers for drug delivery in central nervous system disorders

"GRIEG" research project NCN 2019/34/H/ST5/00578 [2021-2024]
(Project leader: Professor Piotr Warszyński)

Prevention and treatment of neurodegenerative diseases and stroke-related brain damage are major and unresolved problems of contemporary medicine. Despite the progress in understanding molecular mechanisms of neuronal injury and preventing them, few neuroprotective substances are used in the clinic, and their efficiency in treating stroke and neurodegenerations is unsatisfactory. One of the significant limitations to current neurodegenerative disease treatment is an inefficient delivery of neuroprotective drugs to the affected part of the brain and difficulties in the diagnosis if the drug is well addressed, i.e., if it reaches the targeted organ. Theranostics is a new medical branch that joins therapeutic and diagnostic functions in one entity. The application of nanotechnology in theranostics will allow the engineering of drug carriers to simultaneously deliver therapeutic components and possess diagnostic functions.

The main objective of the project is to develop a new strategy to deliver neuroprotectants by applying theranostic nanocarriers for neuroprotective drugs, which are able to cross the blood-brain barrier without imposing side effects on its normal function and can be detected in a given part of a brain by Nuclear Resonance Imaging (MRI). Three Polish scientific institutions, Jerzy Haber Institute of Catalysis and Surface Chemistry of the Polish Academy of Sciences, Jerzy Maj Institute of Pharmacology PAS, Henryk Niewodniczański Institute of Nuclear Physics PAS and two Norwegian, SINTEF Oslo and Oslo University Hospital participate in the project consortium.

In 2024, the project came to an end. We have developed a new strategy for delivering neuroprotective drugs (e.g., carnosic acid) using nanocarriers capable of crossing the blood-brain barrier. Moreover, the developed nanocarriers can be localized by nuclear resonance imaging (MRI) or optical methods using fluorescence. Nanocarriers of neuroprotective substances with emulsion or polymeric cores containing active substances were formed by sequential adsorption of charged polymers functionalized with contrast agents for fluorescence or MRI. In addition, the synthesis method of PEG-stabilized CeO2/Gd nanoparticles with neuroprotective activity was developed. The average size of the developed theranostic nanocarriers was about 150 nm, and they showed stability under physiological-like conditions. The developed nanocarriers were provided to project partners for testing in the *in-vitro* and *in-vivo* models to confirm their neuroprotective properties and ability to overcome the blood-brain barrier. The results obtained in the project provided valuable insights into further pathways for the development of theranostic nanocarriers.

"Polonez Bis" Research Projects of the National Science Centre

1. Simple methods for the syntesis of future, unconventional plasmonic materials with mono-atom and bimetallic centers for sustainable H_2 production and CO_2 reduction

"Polonez Bis" research project NCN 2022/47/P/ST4/03412 [2024-2026]
(Project leader: Priti Sharma PhD)

1. Manuscript Submission entitled "Gram Scale Microwave-Assisted Synthesis of High-Density Fe Single Atomic Sites implanted N-Graphene for Enhanced Oxygen Reduction Reaction" has been received by journal Small. The manuscript number for submission is smll.202501182. In our present studies, we have successfully formulated iron single-atom catalysts supported by nitrogen-doped graphene (FeSA-NG) via in-situ microwave-assisted methods. The precise control of synthesis parameters facilitated by microwave irradiation resulted in the homogeneous dispersion of Fe atoms on the nitrogen-doped graphene matrix high density Fe SA sites (14/05 Tue. % AAS). Electrochemical evaluations reveal that the FeSA-NG catalysts exhibit superior ORR activity, with a higher onset potential (~0.98 V) and half-wave potential (~0.89 V) compared to commercial platinum-based catalysts. The comprehensive physicochemical characterization of FeSA-NG with various Operando Mechanistic Studies including HAADF-STEM and Synchrotron-based X-ray absorption fine structure spectra, Fourier-transform FT-EXAFS spectrum, XANES spectrum and Wavelet transform (WT) EXAFS spectra validate the successful uniform formation of Fe single atoms coordinated to the N doped graphene network successfully. Our findings highlight the potential of microwave-assisted synthesis as a scalable and efficient method for producing high-performance single-atom catalysts, positioning iron single-atom FeSA-NG catalysts as promising alternatives to platinum for ORR applications in sustainable energy technologies.

2. Priti Sharma communicating the MS entitled 'Sterically Challenging Regioselective Diboration and Hydroboration Reactions with Single-Atom Platinum Catalyst.'

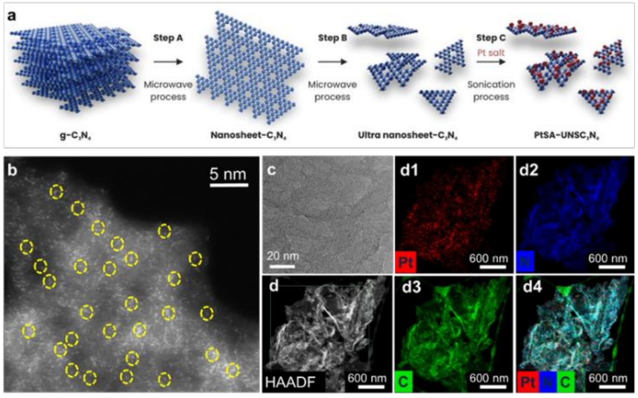


Figure 1. (a) Schematic representation of the synthesis protocol for Pt1-UNSC3N4 (b) STEM image (5 nm scale) highlighting Pt single atoms dispersed on C3N4 sheet, with isolated single atoms marked by yellow circles, (c) TEM image (20 nm scale) showing no evidence of Pt nanoparticles or aggregates, (d) HAADF-TEM image with HAADF-STEM elemental mapping illustrating the distribution of Pt (d1), N(d2), C(d3) elements individually and collectively (d4).

In this study, we report the scalable synthesis of heterogeneous platinum single-atom catalysts (Pt-SACs) supported on ultra-fine graphitic carbon nitride nanosheets using a rapid microwave-assisted technique. These catalysts exhibit excellent regioselectivity and chemoselectivity and outstanding performance in the selective diboration of alkenes, achieving precise 1,2-diboration under remarkably mild conditions, even at room temperature.

"Miniatura" Research Projects of the National Science Centre

1. Thermosensitive Water-in-Water Emulsions for Biomedical Applications Stabilized by Copolymers

"Miniatura" research project NCN 2023/07/X/ST4/01335 [2023-2024]
(Project leader: Andrzej Baliś PhD Eng.)

The synthesis of PNIPAM was optimized through controlled RAFT polymerization. The effects of various factors on polymerization kinetics, monomer conversion degree, and molecular weight were determined. Below are molecular weight distributions (GPC data) for the synthesis where fractions were collected approximately every 2 hours (Fig. 1) and, on the right (Fig. 2), the kinetics of monomer concentration decrease (1H-NMR data) indicative of chain growth. An interesting observation is that the polymerization only began after 4 hours. It is possible that the initial stage of RAFT, involving the formation and propagation of radicals from the initiator (AIBN), did not result in significant macrochain growth. Only the second stage of RAFT, occurring after 4 hours and involving radicals derived from the Chain Transfer Agent (CTA), led to a substantial increase in macromolecule size. It was demonstrated that nearly all the monomer polymerized after 22 hours. The resulting polymer had an average molecular weight Mn of 40 kDa with a reasonably low dispersity M_w/M_n=1.19

The possibility of attaching a second block to the purified PNIPAM polymers (hydrophilic - MAA and hydrophobic - SMA) was investigated. Unfortunately, all five attempts were unsuccessful (GPC data). The purified samples were characterized using TGA to examine differences in the shapes of the thermal decomposition curves. All polymer and copolymer samples exhibited the greatest mass loss dynamics between 350–450 °C, with no significant differences among them. In the course of the study, a successfully synthesized first block sample (PNIPAM) was used to test a system for analyzing emulsion separation with temperature control. The separation of example PEG-DEX emulsions with the addition of 0.2% PNIPAM polymer and the same emulsion sample without PNIPAM as a control were carried out. The use of PNIPAM without a block of hydrophilic/hydrophobic chains did not achieve the intended scientific objective—the effect of spontaneous emulsion formation above LCST. Instead, the upper phase, where 100% of the PNIPAM was found after emulsion separation, changed from slightly cloudy to completely opaque white, not transmitting light. Among the successes of the grant, it is worth noting the development of a reproducible synthesis method for the thermosensitive block using RAFT, the characterization of the obtained emulsions using confocal microscopy stained with FastGreen/BLG nanogels and improving the emulsion separation testing system with a temperature module.

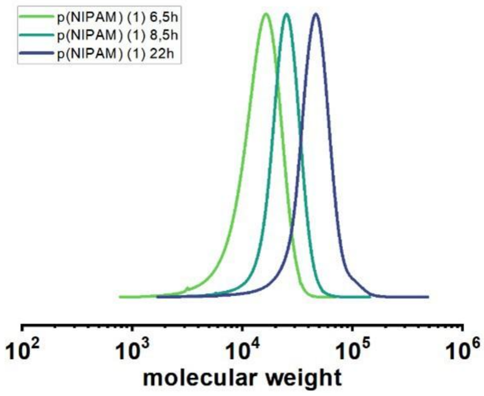


Fig. 1. GPC data of p(NIPAM) chain growth in time

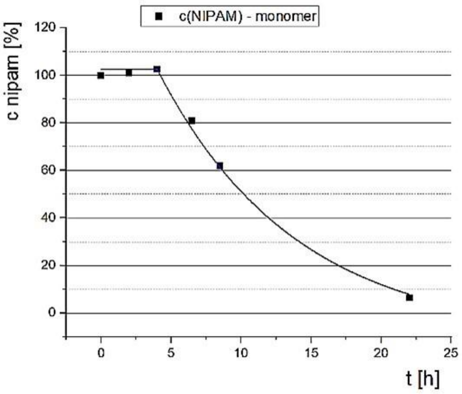


Fig. 2. Analysis of kinetics of RAFT reaction of P-NIPAM measured as loss of concentration of monomer (1H-NMR)

2. Stability and physicochemical properties of foams formed from solutions of surface active substances and polyelectrolytes

"Miniatura" research project NCN 2023/07/X/ST11/00476 [2023-2024]
(Project leader: Dominik Kosior PhD Eng.)

The primary goal of the project was to investigate the potential application of bio-polylectrolyte and bio-surfactant mixtures in the formation and stabilization of foam films. The choice of the polyelectrolyte/surfactant (PE/S) system was guided by the properties of the components, with particular emphasis on biodegradability, biological activity (antiseptic), and biocompatibility. Using complementary experimental techniques for studying foam films under both static and dynamic conditions, the synergistic and antagonistic effects of polyelectrolyte/surfactant systems on the stability of foam films were examined.

The work was focused on the impact of various polyelectrolyte/surfactant mixtures on the stability of the foam films produced. Synthetic polypeptides were selected as the polyelectrolytes: (i) cationic poly-L-lysine (PLL) and poly-L-arginine (PARG), and (ii) anionic poly-L-glutamic acid (PGA). As the second component of the PE/S systems, two bio-surfactants were chosen: cationic ethyl lauroyl arginate (LAE) of high purity and moderately anionic rhamnolipid (RH), a mixture of mono- and di-rhamnolipid homologs.

Through measurements of surface tension and foam film lifetimes under static conditions, it was demonstrated that the optimal concentration of polyelectrolytes (PLL, PARG, and PGA) was 100 mg/L. This polyelectrolyte addition to the tested surfactants significantly reduced surface tension and avoided phase separation effects up to concentrations of 1×10^{-3} M for LAE and 600 mg/L for RH. These concentrations were critical thresholds, as indicated by the appearance of turbidity and significantly shortened foam film lifetimes.

Even more intriguing, due to their similarity to real foam production conditions, were standard foamability studies of the tested PE/S systems using the dynamic foam analyzer (DFA) method. The addition of PGA to LAE markedly improved the foamability properties of the system. In contrast, the PLL/RH and PARG/RH systems showed much weaker effects. For the PGA/LAE system, the surfactant is strongly cationic, whereas in the PLL(PARG)/RH systems, rhamnolipids exhibit weak anionic properties at neutral pH (5.8), resulting in much weaker interactions with the polypeptide compared to the PGA/LAE mixture. Altering the pH from acidic to basic (pH 10) improved foam stability due to the enhanced anionic character of RH; however, turbidity appeared at much lower surfactant concentrations. The results obtained in the foamability tests were interpreted in the context of studies on the drainage of thin liquid films under dynamic conditions (dynamic fluid-film interferometry, DFI). The use of interferometric methods enabled measurements not only of the drainage times of thin liquid films but also of their thickness as a function of time. The resulting drainage kinetics were compared with theoretical drainage curves based on existing models in the literature. It is worth noting that the foam lifetimes observed in DFA experiments correlated well with the drainage rates of thin foam films measured using interferometric methods.

The scientific project was concluded in 2024 with the submission of a final report to the National Science Centre. The outcomes of the work included the publication of two research articles in scientific journals: *MDPI Materials* and *ACS Langmuir*.

3. Influence of nanocrystallization of Mg on structure and corrosion resistance of phosphate conversion coatings for biomedical applications

"Miniatura" research project NCN 2023/07/X/ST11/00036 [2023-2024]
(Project leader: Konrad Skowron PhD Eng.)

As a part of the work conducted in the 2024 reporting year, the project leader successfully realized all planned research objectives of the Miniatura VII grant were. Based on the conducted research, the final report on the completed work, numbered 80525, was submitted to the National Science Centre (NCN).

The main scientific finding of the conducted research is that microstructural changes induced by the surface mechanical attrition treatment (SMAT) significantly alter the behavior of pure magnesium in corrosive environments, increasing its reactivity. This enhanced reactivity promotes the rapid formation of a dense layer of corrosion product, which then protects the Mg surface from further corrosive. The increased reactivity of Mg substrates also significantly affects the chemistry of conversion treatment, improving the morphology and corrosion protection performance of phosphate layers on their surfaces. The obtained results indicate that the surface structures achieved through the SMAT facilitates the formation of higher-quality phosphate layers on magnesium.

4. Development of a methodology for obtaining foamed hydrogel material: description of forming processes and characterization of obtained structures

"Miniatura" research project NCN 2024/08/X/ST8/00054 [2024-2025]
(Project leader: Sonia Kudłacik-Kramarczyk PhD Eng.)

Formulations of foamed hydrogels based on Ecogel™, glycerin, gelatin, and Tween 80 were developed. The experiments included various proportions of components, with particular focus on varying the Tween 80 content in the range of 0% to 50% by weight relative to glycerin. The material formation process relied on intensive mixing using a homogenizer (8500 rpm, 5 min) to achieve uniform structures. FT-IR spectroscopy analysis revealed characteristic bands associated with functional groups such as carbonyl, methylene, and ester groups. These findings confirm the modifying influence of Tween 80 on the chemical structure of the hydrogels. Importantly, the analysis did not show bands indicative of potentially toxic by-products, underscoring the chemical safety of the synthesis process. Microscopic studies demonstrated that increasing the Tween 80 concentration enlarged the diameter of air bubbles within the hydrogel structure. This feature may enhance application properties, such as spreadability and skin absorption. Materials with higher Tween 80 content exhibited greater water contact angles, indicating a more hydrophobic nature. Surface energy analysis confirmed a predominance of the dispersive component in samples containing Tween 80. Materials containing Tween 80 showed a greater tendency to release water into the environment, whereas samples without Tween 80 effectively retained moisture. Stability studies using a multiscan system demonstrated that the addition of Tween 80 stabilizes the foam structure, reducing the tendency for phase separation. Storing the samples for 24 hours at 6°C further improved their stability.

Figure 2. Compilation of FT-IR spectra of oleogel sample without Tween and with the highest content of this emulsifier (**up**) and compilation of FT-IR spectra of all test samples (**down**). The experiment was conducted in triplicate and the presented data represent a representative result from the measurements.

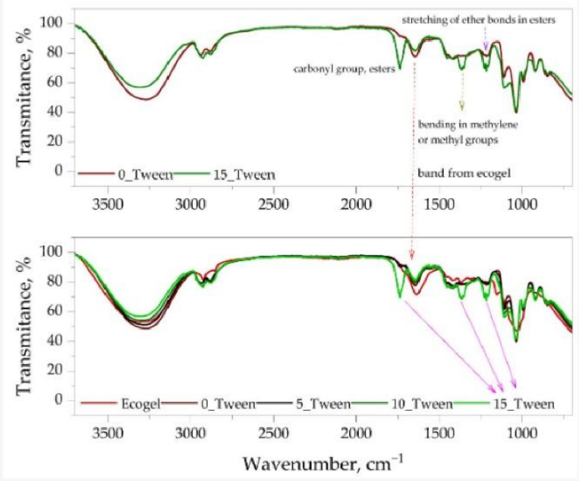


Figure 8. Results of the stability analysis of the oleogels directly after their synthesis (30 min measurement). The experiment was conducted in triplicate and the presented data represent a representative result from the measurements.

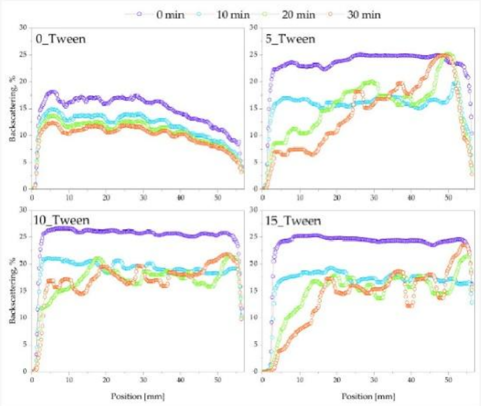
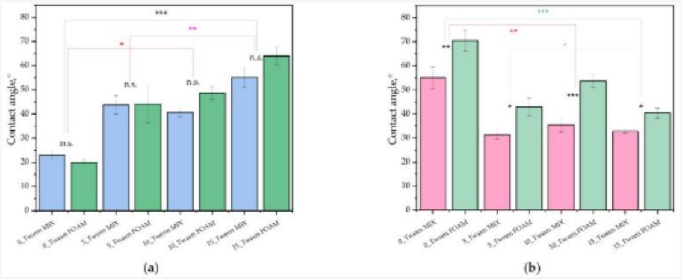


Figure 4. Contact angles of oleogel samples (both mixtures and foams). Panel (**a**) represents measurements for water and panel (**b**) represents measurements for diiodomethane. Error bars represent standard deviations ($n = 3$). Statistical significance (p-values) between MIX and FOAM for each concentration is indicated above the bars (n.s. = not significant, * $p < 0.05$, ** $p < 0.01$, for *** $p < 0.001$). Significant differences between concentrations (Tukey's HSD) are marked with asterisks above the corresponding groups.



Research Projects of the National Centre for Research and Development

1. Development of a functional form of application of sugar esters of fatty acids based on bacterial polyhydroxyalkanoates that inhibit cancer cell growth

„TANGO" research project NCBiR TANGO-V-A/0013/2021 [2022-2024]
(Project leader: Associate professor Maciej Guzik)

The project focused on the development of an innovative nanoemulsion containing the chemotherapeutic agent SN-38. The aim of the project was to enhance the effectiveness of cancer therapies by utilizing a nanoemulsion that enables precise drug delivery to cancer cells while minimizing its impact on healthy tissues and reducing the side effects of treatment.

During the project, a stable nanoemulsion was obtained, maintaining its properties for at least five months under refrigeration (6–8°C). In vitro studies demonstrated the increased therapeutic efficacy of SN-38 in combination with the nanoemulsion compared to the standalone drug. In vivo results on colorectal cancer HCT116 xenograft models confirmed the synergistic effect of the formulation, allowing comparable therapeutic outcomes at lower doses of SN-38. The addition of the emulsion enabled a fivefold increase in tumor growth inhibition compared to SN-38 alone at low concentrations.

The project results open new possibilities in personalized oncology, enabling the development of more effective and safer cancer treatments.

The project's achievements culminated in a patent application for a "Nanoemulsion composition and its use in cancer treatment, particularly colorectal cancer" (application no. P.447923), submitted to the Polish Patent Office on March 4, 2024. This application underscores the innovation and commercialization potential of the developed technology, enhancing its market value and prospects for implementation.

Exchange of PhD Students and Academic Staff Programme Projects of the Polish National Agency for Academic Exchange

1. International Scholarship Exchange of PhD Candidates and Academic Staff

Project NAWA BPI/PRO/2024/1/00025/U/00001 PROM [2024-2025]
(Project coordinator: Professor Tomasz Borowski)

In November 2024, competition regulations were prepared, and in December 2024, two competitions were announced for scholarships to finance the costs of short-term forms of education abroad for doctoral students and staff of IKiFP PAN, and for doctoral students from abroad for internships at IKiFP PAN.

2. Nanocarriers for Drug Delivery with Photosurfactants and Polyelectrolyte

Project NAWA BPN/BDE/2024/1/00031 [2024-2026]
(Project leader: Marcel Krzan DSc)

The aim of the project is to develop the foundations of technology for manufacturing interactive emulsion-based drug nanocarriers based on mixtures of photosurfactants with polyelectrolytes. Light-responsive nano- and micromaterials have considerable applications in various areas, such as energy storage, molecular electronics, biomimetics and drug delivery. Incorporating surfactants with variable surface activity controlled by UV light as the basis of a dispersed system responsive to stimuli is the key to inducing spatially precise interaction in practical applications. For example, simple azobenzene-based photosurfactants have been used to create complex coacervates to capture and release biomolecules. Nowadays, great efforts are being put into designing nanocarriers for drug delivery induced by external stimuli such as light or magnetic field due to site-specific administration, possibility of dose adjustment, minimal interference with healthy tissues and strict spatiotemporal control. Photoisomerization is one of the simplest drug release mechanisms using photoreactive carriers, involving encapsulating the desired drug using the E-isomer of the photosurfactant and subsequent release after photoisomerization into the Z-isomer. The change of polarity and hydrophilicity of the isomer mediates the release via E/Z photoisomerization. However, the Z-isomer of most azobenzene amphiphiles has low thermal stability. Therefore, long-term or periodic high-intensity UV irradiation is necessary to obtain the drug release effect. Photoreactive nanocarriers are needed to provide prolonged release of the encapsulated drug at low intensities and short exposure times to UV light to obtain better control over the UV-induced drug release kinetics. We propose using arylazopyrazoles (AAPs), which are photosurfactants with longer Z-isomer half-lives of several days to weeks, provided that the solution pH is high enough. Under these conditions, almost complete E/Z photoswitching is possible at photostationary states > 90% and large changes in the dipole moment after photoisomerization. Due to the increased stability of the Z-isomer, AAPs as a photoreactive scaffold can allow the fabrication of suitable drug carriers in which the induction of drug release will occur at shorter exposure and lower UV intensity. Introducing photoswitchable surfactants, especially those based on arylazopyrazole AAP, into the system can significantly accelerate the development of drug delivery systems. Similar surfactants offer unique properties that allow for controlled drug release, providing an innovative approach to drug delivery. The project focuses on using photoswitchable AAP surfactants and their mixtures with polyelectrolytes. These mixtures, in addition to their significant potential for controlled drug delivery, can also be used as emulsifiers.

Horizon Programme

1. IPERION HS - Integrating platforms for the European Research Infrastructure on heritage science

Horizon 2020 Programme no. 871034 [2020-2024]
(Project leader: Professor Łukasz Bratasz)

The goal of the IPERION HS project is to establish and operate a distributed pan-European research infrastructure, strengthening key national research facilities of recognised excellence in heritage science. Heritage science is a young and cross-cutting scientific domain embracing a wide range of research disciplines enabling a deeper understanding of the past and improved care for the future of heritage. Since 2016, heritage science is included in the European Strategy Forum on Research Infrastructures Roadmap as one of the strategic areas in the domain of Social Sciences and Humanities, where it is represented by the European Research Infrastructure for Heritage Science. IPERION HS will provide the advanced community of heritage science a further level of pan-European integration, in view of the establishment of European Research Infrastructure for Heritage Science. The project is a further step towards a unified scientific approach to the most advanced European instruments for the analysis, interpretation, preservation, documentation and management of heritage objects in the fields of art history, conservation, archaeology and palaeontology. The IPERION HS project will be offering not only trans-national access to a wide range of high-level scientific instruments and methodologies, but for the first time, it will focus on the needs of preventive conservation in museums and other memory institutions. One of the key actions will be the development of the Preventive Conservation Digital Platform (PCDP) enabling users to model and plan future risk scenarios and adjust preventive conservation measures accordingly. The PCDP will be the first step for the heritage science community to actively support the European Open Science Cloud initiative to build a competitive data and knowledge economy in Europe.

In 2023, the project continued to thoroughly test and optimize the decision support tools for practitioners, museum professionals and facility owners developed in previous years. The tests covered all created tools and the data uploading module. Detected software shortcomings were improved and corrected based on discussions with project partners. Work was also undertaken on the integration of COMSOL Multiphysics software for finite element calculations with the HERIe platform. Communication in Java was initially tested for several versions of sample files generated by the "application compiler" module of COMSOL. The work will continue as part of the agreed project with the Getty Foundation.

An important element of the project was also the dissemination of knowledge about the HERIe platform among end users. Organized two international workshops: one online with the Rijksmuseum and one in Ottawa at the Canadian Conservation Institute.

2. NanoPaInt - Dynamics of dense nanosuspensions: a pathway to novel functional materials (nanoPaInt)

Project H2020-MSCA-ITN-2020 nr 955612 [2021-2024]
(Project leader: Professor Piotr Warszyński)

Nanotechnology is an innovative trend in the development of science and technology, which creates opportunities for progress in many areas of our lives. It includes the design, characterization, production, and application of structures and devices at the nanometer scale in many areas of our life. The products of nanotechnology are extremely useful because of their specific physical as well as chemical properties. In the last few years, metallic nanoparticles (NPs) have attracted much attention due to their small dimension and large surface-to-volume ratio, which determine their specific properties that differ from their macro-scale counterparts. NPs are interesting not only from a scientific point of view but also from the possibilities of their application in various industries such as the cosmetics, textile, or pharmaceutical industry. One of the applications of metallic nanoparticles, due to their low melting point and high conductivity, is the printed electronic industry. Such NPs are used as a main component of conductive materials (inks or pastes) for producing, for example, solar cells, thin-film transistors, printed circuit boards, transparent conductive electrodes, flexible displays, electrochromic devices, and touch screens. The NanoPaInt network aims to understand, predict, model, and apply the strongly interacting nanoparticle-laden systems out of equilibrium. The training aim of the NanoPaInt network is to support the career development of young researchers both in academic and non-academic sectors and to train a new generation of creative and innovative early-stage researchers (ESRs) through the experience of independent and interdisciplinary research, participation at local and network-wide training activities and secondments.

During the research carried out in 2023 as part of ESR 13, the optimization of the synthesis process of copper@silver nanowires with a core@shell structure (Cu@Ag NWs) and their use in the production of conductive materials was continued. In the first stage of the research, copper nanowires were obtained as the core of "core@hell" structures. In the second stage, a silver shell was formed on the obtained nanowires as a result of the transmetalation reaction. Cu@Ag NWs with optimal physicochemical properties (morphology and copper resistance to undesirable oxidation processes) were obtained in the presence of ascorbic acid, which has both reducing and antioxidant properties, which was confirmed using CONFOCAL and SEM microscopies and the XPS method. SEM images (Fig. 1) clearly indicate that after adding ascorbic acid (AA) after 3 minutes of adding $AgNO_3$, the obtained silver shell was more uniform (Fig. 1B) compared to the one formed after adding AA directly after the silver coating precursor (Fig. 1A). In the next stage of the research, pastes based on the obtained Cu@Ag NWs were produced, which in the next year of the project implementation will be used for the obtaining of conductive tracks or coatings.

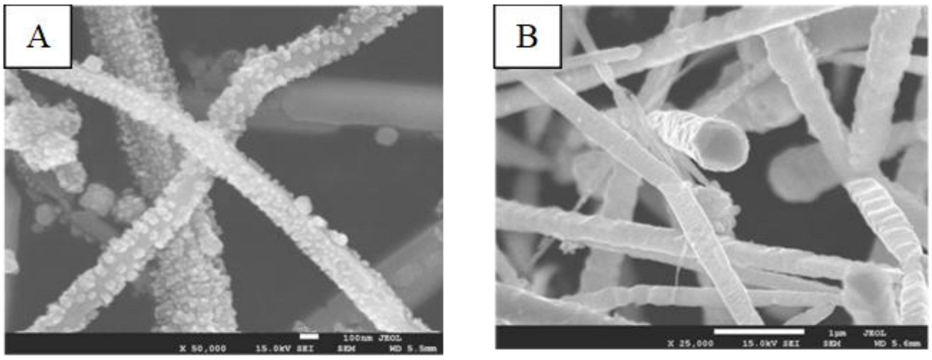


Fig 1. SEM images of Cu@Ag NWs in the presence of ascorbic acid, which was added immediately (A) and after 3 minutes (B) of adding $AgNO_3$.

3. NewCat - Teaching Lytic Polysaccharide Monooxygenases to do Cytochrome P450 Catalysis

Horizon Europe EIC-PATHFINDER nr 101046815 [2022-2025]
(Project leader: Professor Tomasz Borowski)

EU Green Deal strategy and Climate Mitigation mission require the application of radically new technologies non existing yet on the market. The EIC Pathfinder call goes beyond what is already known and welcomes the high-risk/high gain and interdisciplinary cutting-edge science collaborations that underpin technological breakthroughs.

The NewCat project was inspired by nature and aims to provide a universal platform facilitating organic synthesis via clean, organic solvent-free and low energy consumption processes. The required knowledge is granted by a combination of the state-of-the-art experimental and computational methods in the area of protein engineering, enzyme catalysis, nanomaterials and large-scale protein production delivered by partners of the consortium.

The project is realized by a consotrium of four partners: Norwegian University of Life Science, Norway, a coordinator, Technical University Graz, Austria, ICSC PAS, Poland and Silesian University of Technology, Poland. The team at ICSC PAS contributes to the project its expertise in the field of computational studies on metalloenzymes as well as protein crystallography.

The work carried out in 2023 focused on computational research, protein production, enzyme crystallization (LPMO of unknown structure and LPMO in previously unstudied complexes), study of protein-ligand interactions of selected LPMOs with a selected list of potential ligands using the microscale thermophoresis method, MST).

4. GoGreen: green strategies to conserve the past and preserve the future of cultural heritage

Horizon Europe HORIZON-WIDERA nr 101060768 [2023-2026]
(Project leader: Professor Łukasz Bratasz)

The GoGreen project's core objective is to initiate a green revolution by reinventing conservation practices towards environmentally friendly solutions. The starting point is that current theory and practices employed for remedial and preventive conservation do not sufficiently consider the environmental impact of conservation, display and storage. The ambition of the project is to take action in both conservation strands by promoting, on the one hand, green materials and methods for remedial treatments, and on the other, the application of preventive conservation strategies which take into consideration environmental impact. As the widening partner, The IKiFP work focuses on evidence-based preventive measures for sustainable strategies of indoor environment control in the cultural heritage sector. Rational decisions regarding climate control are currently impossible as environmental control requirements for specific historic material types are often unavailable. To address the challenge, GoGreen develops new damage functions for glass, metal, ceramic, limestone, wall paintings on lime-based plaster and paint layers in easel paintings that describe physical degradation models of vulnerable objects.

A set of samples of the animal glue-based grounds - the gesso - that represent one of the most brittle materials constituting painting to extend known mechanical parameters to high temperatures and humidity levels using time-temperature superposition rule was prepared by the research team at IKiFP. The gesso was characterised by the Dynamic Mechanical Analyser. Using the DMA results, the experimental horizontal shift factors aT were used to find correspondence between temperature and time. The linear fitting of the shift factors plotted against temperature was performed using the Arrhenius equation. The results show that the shift factors measured for gesso follow the Arrhenius equation and allow the calculation of an activation energy of 88±6 kJ/mol.

Further, several campaigns of monitoring climate-induced micro-damage in selected original objects displayed in real-world conditions were initiated to validate the modelling outcome. The objects include illuminated parchment sheets from the archive of the Dominican Monastery in Krakow (monitoring of irreversible curling of parchment), a wooden sculpture from St. Maty's Basilica in Kraków loaned to an exhibition in the Wawel Royal Castle in Krakow (monitoring the micro-fracturing of wood with the use of acoustic emission technique).

Another material investigated is unstable glass. Two compositions, representative of historic glasses known as unstable owing to a low content of CaO+MgO and a high content of alkali oxides, were selected. The samples were artificially aged for several days at a temperature 80 ^{o}C and relative humidity (RH) 85%. During the decrease of RH, the acoustic emission technique (AE) was applied to follow the microcracking development. The first test showed that AE can be successfully used for that purpose despite the glass's very low fracture toughness. The test will be continued in the coming year to determine critical levels of RH at which development of microcracking starts.

Other International Research Projects

1. Bioplastics upcyclig loop (BioPolyCycle)

ANSO-CR-PP-2022-01
(project leaders: Associate professor Maciej Guzik DSc)

Recent studies have advanced a chemobiotechnological method of upcycling biodegradable plastics, focusing on the efficient depolymerization of polyesters such as polycaprolactone (PCL), polylactic acid (PLA), and polyhydroxybutyrate (PHB) into high-value intermediates. These intermediates were subsequently utilized as feedstocks for microbial fermentation to produce biopolymers, particularly PHB. This innovative approach aligns with the circular economy framework, promoting sustainable practices in plastic waste management while addressing critical environmental challenges.

The study employed alkaline hydrolysis with potassium hydroxide (KOH) in methanol to achieve high-yield depolymerization under optimized conditions. Stoichiometric degradation yielded 88–100% conversion efficiency for PHB, PCL, and PLA. The degradation products were characterized using FT-IR spectroscopy and directly applied as feedstocks in bacterial cultures without additional purification.

Screening experiments involving six bacterial strains identified *Cupriavidus necator* B4383 as the most effective candidate for upcycling degradation products into PHB. This strain demonstrated robust metabolic pathways, achieving up to 58% PHB content in dry cell weight when utilizing 3-hydroxybutyric acid as a substrate. Other substrates, such as crotonic acid and 6-hydroxycaprylic acid, also supported significant biopolymer production, albeit at slightly lower efficiencies. Conversely, lactic acid showed limited potential for PHB synthesis.

These findings highlight the feasibility and scalability of integrating abiotic degradation and microbial fermentation in a single framework, showcasing the potential to transform bioplastic waste into high-value biopolymers. This dual-process strategy not only mitigates the environmental impact of plastic waste but also supports the transition toward sustainable material innovation.

2. Organosmectites doped with inorganic cations as adsorbents of controlled hydrophobicity/hydrophilicity

Joint research project of the Polish Academy of Sciences and the Czech Academy of Sciences cooperation PAN-24-22 [2024-2026]
(project leader: Dorota Duraczyńska PhD)

As part of a jointly implemented project, the Jerzy Haber Institute of Catalysis and Surface Chemistry, Polish Academy of Sciences (ICSC PAS), hosted the following researchers from the Institute of Geonics of the Czech Academy of Sciences (IGN): dr. Lenka Vaculíková and dr. Eva Plevová (June 5–6, 2024, and October 9–12, 2024), as well as dr. Mahdi Niktabar (October 9–12, 2024). Meanwhile, dr. Dorota Duraczyńska and dr. Robert Kosydar, researchers from ICSC PAS, visited the IGN, from December 5–12, 2024, while professor Magdalena Oćwieja, participated in the visit from December 5–7, 2024.

During the first visit to ICSC PAS, preliminary studies were conducted on the initial samples, analyzing their morphology and chemical composition using scanning electron microscopy (SEM). This technique enabled the acquisition of detailed surface images of the samples and the identification of their elemental composition, which is crucial for understanding their adsorptive and structural properties. In the meeting, a detailed research plan was developed, encompassing synthesis processes, characterization, and potential applications of the studied samples. Particular attention was given to the methods of synthesizing organo-smectites and techniques for their modification with inorganic cations. A comprehensive physicochemical characterization of the samples was also emphasized to precisely determine their hydrophilic and hydrophobic properties. Additionally, the potential applications of the developed materials as adsorbents in various technological processes were discussed, paving the way for their practical use in industry.

During the second visit to ICSC PAS, studies were conducted on five newly synthesized samples, focusing on their hydrophobic and hydrophilic properties through contact angle analysis. The contact angle, which measures the adhesion of a liquid to the surface of a sample, clearly determines whether a material is hydrophobic (higher contact angle) or hydrophilic (lower contact angle). Additionally, X-ray diffraction (XRD) was used to analyze the crystal structure of the samples. This method allowed the identification of mineral phases and the assessment of grain size and degree of crystallinity, which is crucial for understanding the impact of internal structure on the hydrophobic and hydrophilic properties of materials. The combination of results from contact angle analysis and XRD provided a comprehensive insight into the physicochemical properties of the studied materials. Such data are essential for their further development and potential applications in fields such as materials engineering, nanotechnology, and the chemical industry.

During the visit to IGN, the results obtained at ICSC PAS for five newly synthesized samples were discussed, including analysis using electron microscopy and energy dispersive spectroscopy (SEM/EDS), contact angle measurements, and zeta potential measurements. Additionally, on-site studies were conducted using Raman spectroscopy. Four new samples were also prepared and sent for further analysis at the Jerzy Haber Institute of Catalysis and Surface Chemistry, Polish Academy of Sciences.

3. Project “Educational Mobility” for students and employees

EU Erasmus+ Programme [2014-2027]
(project coordinator: Agata Pomorska Gaweł PhD)

In 2024 the following mobilities were implemented:

a) Student traineeship:
- Joanna Szechyńska University of Ostrava (Czechy)

b) Staff mobility for training:
- Andrzej Baliś– Laboratoire Charles Coulomb (Francja)
- Dzmitry Kharytonau– Lulea University of Technology (Szwecja)
- Konrad Skowron- Lulea- University of Technology (Szwecja)
- Małgorzata Ruggiero – University Erlangen (Niemcy)
- Anna Faruga- University of Belgrade (Serbia)
- Justyna Prajsnar- University of Belgrade (Serbia)
- Agata Wiertel- Pochopień- Czech Academy of Sciences (Czechy)
- Jan Zawała- Czech Academy of Sciences (Czechy)
- Maciej Szaleniec- University of Amsterdam (Holandia)

The effects of the educational mobility included collecting research material for: a doctoral thesis, articles in international scientific journals and conference presentations.

The project will be continued in 2025.

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