

**JERZY HABER INSTITUTE OF CATALYSIS  
AND SURFACE CHEMISTRY  
POLISH ACADEMY OF SCIENCES**



HR EXCELLENCE IN RESEARCH

# **DETAILED RESEARCH REPORT**

**year 2023**



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# **Statutory Research**



## **Catalytic Materials and Processes for Sustainable Development**



## Experimental and computational studies on selected enzymes of application potential

(Professor Tomasz Borowski, Professor Maciej Szaleniec, Anna Kluza PhD, Anna Milaczewska-Kręgiel PhD, Mateusz Tataruch PhD Eng., Zuzanna Wojdyła PhD, Agnieszka Wojtkiewicz PhD, Justyna Andrys MSc)

A chromatographic method was developed to identify the substrate (dihydroxyphenylalanine, DOPA) and product (betalamic acid) of the reaction catalyzed by the enzyme L-DOPA extradiol dioxygenase from *Beta vulgaris* (DODA, L-DOPA 4,5-dioxygenase) using the Ascentic RP-Amide Express column. A reactor test was developed to determine the activity of a homogeneous enzyme, based on which the activity of the non-immobilized enzyme was detected. Covalent immobilization of the BvDODA enzyme was performed on three silica supports of the SBA-15 type having hydroxyl (linker: 3% divinylsulfone), amine (linker: 3% glutaraldehyde) and glycidyl groups. After three hours of immobilization, quantitative protein analysis showed 100% binding of the protein to the carriers. In a 16-hour reactor test, the immobilized enzyme did not show catalytic activity.

For the tH6H enzyme, preliminary studies of the fast reaction kinetics were performed using the stopped-flow method with monitoring of the reaction progress using UV-Vis spectra. Unfortunately, it turned out that the extinction coefficient for the ferryl intermediate was too low to observe its formation and disappearance with the available equipment at the achievable concentrations. For tH6H-Fe(II)- $\alpha$ KG-HYO and tH6H-Fe(II)- $\alpha$ KG-6OH-HYO complexes obtained under anaerobic conditions and for quickly (100 and 500 ms, respectively) frozen reaction mixtures obtained by mixing solutions of these complexes with oxygenated buffer, Mossbauer spectra were measured. Deconvolution of the obtained spectra confirmed the presence of a ferryl intermediate and provided the first spectroscopic parameters for tH6H-Fe(IV)O-SUC-6OH-HYO. Stopped-flow and rapid freeze quench studies were carried out in cooperation with the group of prof. A. Osyczka (WBBiB UJ), and Mossbauer's measurements were performed by prof. J. Korecki.

For the tCODM enzyme, a crystal structure was obtained for the complex of the protein with Ni(II), NOG and protopine, which is the first available structure providing valuable insight into substrate binding by plant O-demethylases. This structure was the starting point for computational studies, which included: molecular dynamics simulations for the tCODM-Fe(II)- $\alpha$ KG-protopine and tCODM-Fe(IV)O-SUC-protopine complexes and QM/MM calculations for the protopine hydroxylation reaction. The calculated free energy profiles suggest that the activation reaction of the C-H bond takes place in the so-called  $\pi$  channel, which leads to the formation of an intermediate in the excited state. The next stages of the reaction are deexcitation to the ground state and recombination of the carbon radical with the OH group.

For the UGT270 enzyme, a series of molecular dynamics simulations were performed for several variants of the enzyme: the apo form, the complex with UDP-glucose and double complexes with UDP-glucose and: campferol, naringenin or quercetin. The analysis of the obtained trajectories made it possible to: a) propose the orientation of substrate molecules in crystal structures, b) determine the mobility of the loops that close the active site of the enzyme, depending on the loading of ligands, c) determine the binding energy of the ligands for several possible orientations within the binding site.

## **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., Wojciech Snoch PhD)

Deep eutectic solvents (DES) form one of classes of ionic liquids. For the first time they were reported under that name in the beginning of the 21<sup>st</sup> century. DESs are formed as a result of the interaction between at least two chemical compounds – one of them acts as a hydrogen bond donor (HBD), and the other as its acceptor (HBA). The interaction between these substances through hydrogen bonding significantly alters the physicochemical properties of the obtained mixture compared to the properties of its pure components. Among the chemicals capable of forming DESs, natural compounds are particularly important. DESs based on natural compounds have gained their own moniker – NADES (Natural Deep Eutectic Solvents). These mixtures are produced using organic acids, amino acids, amines and a variety of other natural compounds. In reported studies the suitability of biopolymers obtained through bacterial fermentation as a source of components for preparing NADES-type liquids was investigated. Biopolymers are known to be biocompatible and biodegradable. Their use as a source of 3-hydroxyacids would allow to use potential waste or used products made from biopolymers as substrate for preparation of new, useful products.

**The aim of the work** was to determine usefulness of bacterial biopolymers as a source of chiral 3-hydroxyacids for application as hydrogen bond donors (HBD) in preparation of natural deep eutectic solvents (NADESs). Three bacterial polymers produced via bacterial fermentation were selected as substrates for performed studies:

- 1) polyhydroxybutyrate (PHB) – produced by *Zobellella denitrificans* fed with glycerol,
- 2) polyhydroxyoctanoate (PHO) – produced by *Pseudomonas putida* KT2440 from octanoic acid,
- 3) polihydroksynonanoate (PHN) – produced by *Pseudomonas putida* KT2440 from nonanoic acid.

For each of selected polymers a series of processes were carried out to produce a mixture of natural 3-hydroxyacids. Yield of the obtained acids was calculated as ratio between mass of monomers mixture and parent biopolymer. Mixtures of monomers were analyzed using GC and HPLC methods as well as mass spectrometry. In the case of monomers from PHO and PHN the final yield of the product after completing the purification, drying, and extraction procedures was c.a. 86%. Attained yield of 3-hydroxybutyric acid was around 30%. Further studies focused on the selection and optimization of synthesis procedure and investigation of elementary steps of 3-hydroxyacids preparation from PHB. Reaction mixtures were sampled at each stage of the process and subjected to chromatographic analysis. Depending on the chosen processing method the determined product yield ranged from 30 to 65%. The optimization of this process will be continued to maximize the yield of R-3-hydroxybutyric acid monomers. The obtained mixtures of 3-hydroxyacids and commercial choline chloride were used to create NADES by mixing these components in various HBD:HBA ratios, which followed preliminary characterization of the newly synthesized systems.



## New peroxy and polyoxy 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 purpose of our research was the synthesis, structural, physicochemical studies and determination of the catalytic activity of new polyoxomolybdates in the oxidation of hydrocarbons. Polyoxomolybdates are an inorganic compounds of the POM type, which in their three-dimensional structure combine oxygen ions with molybdenum as a transition metal in the highest oxidation state. The composition of the polyoxomolybdate molecule can be adjusted in a special way which allows for the introduction of those elements which are necessary to obtain desired properties. Due to this structural diversity, polyoxomolybdates are widely applied in such fields as medicine, materials science and catalysis.

The current research was aimed at the synthesis of mono-, di-, tri-molybdates and molybdates with Anderson structure modified with transition metals such as Ni, Zn, Co, Cu. Polyoxomolybdates of methylbenzylamine were also obtained. The synthesized materials were characterized by tg/DSC thermal analysis and X-ray diffractometry XRPD to determine the phase compositions.

The obtained catalysts were applied in the liquid phase oxidation of cyclooctane with molecular oxygen in a stainless steel reactor, at 120°C, under 10 atm air pressure for 6 hours. All the prepared catalysts were active in the studied reaction. In the addition to cyclooctanone and cyclooctanol, suberic acid was also formed in the course of the reaction. The highest activity was observed for zinc and cobalt trimolybdates, while Cu trimolybdate showed the lowest catalytic activity. Both methylbenzylamine mono- and octamolybdate demonstrated moderate catalytic activity in the studied reaction, with MBA-MONO having a much higher yield to ketone (42.9%) compared to MBA-OCTA (36.9%).

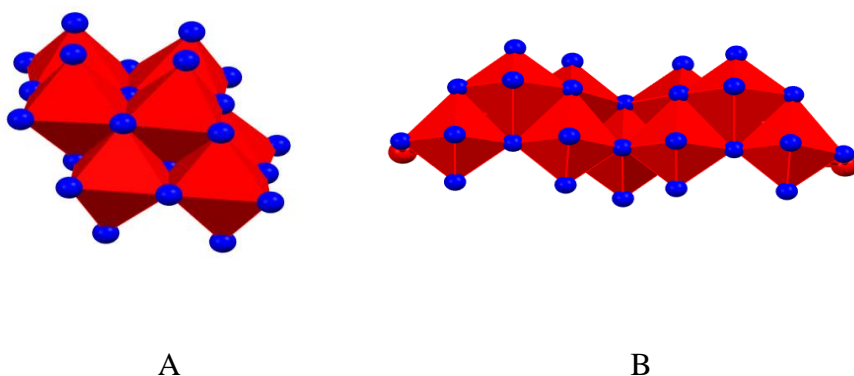


Fig. 1. Octamolybdate (A) and trimolybdate (B) anions observed in investigated polymolybdates.

## Studies of structural effects and influence of support on the activity of bimetallic copper-nickel catalysts

*(Associate professor Dorota Rutkowska-Żbik, Professor Małgorzata Witko, Associate Professor Renata Tokarz-Sobieraj, Anna Micek-Ilnicka DSc, Michał Śliwa PhD, Katarzyna Samson PhD Eng., Małgorzata Ruggiero-Mikolajczyk PhD, Łukasz Kuterasiński PhD, Mariusz Gackowski PhD, Robert Kosydar PhD, Erwin Lalik PhD, Małgorzata Zimowska PhD, Jerzy Podobiński MSc, Natalia Ogródowicz MSc, Joanna Kaim MSc)*

The aim of this year studies was to check how the type of the oxide-type support influences the properties and catalytic activity of copper - nickel containing materials in low temperature hydrogenation of furfural in the gas phase.

As the support for the active phase, the following oxides were used: SiO<sub>2</sub> (amorphous silica, SSA = 213 m<sup>2</sup>/g), CeO<sub>2</sub> (SSA = 2,60 m<sup>2</sup>/g) and ZrO<sub>2</sub> (SSA = 0,87 m<sup>2</sup>/g). The metallic copper-nickel phase was introduced via wet impregnation using copper(II)nitrate and nickel(II) nitrate. Next, the samples were dried at 120 °C for 10 h and calcined at 500 °C for 3 h in the air flow to obtain catalysts containing oxide-based species. The resulting materials, in which copper to nickel content was equal 1:1, while the metal content reached 2 and 5 wt. %, were characterised by a set of physico-chemical methods: XRD, H<sub>2</sub>-TPR, N<sub>2</sub>-physisorption, XRF and SEM/EDS. The catalytic reaction was performed in the fixed-bed flow reactor. The products and substrates were analyzed using gas chromatograph equipped with FID detector. For typical measurement, the 150 mg of catalyst was mixed with quartz and placed in the reactor. Next, the sample was reduced (at temperature as determined by H<sub>2</sub>-TPR) in the mixture of 20% H<sub>2</sub> in Ar and temperature was lowered to 120 °C. The thermostated bubbler at 54.5 °C was used to generate vapors of FAL. The volume ratio of furfural to hydrogen in the reaction stream was 1:10. The catalytic reaction was carried out at 120°C, 160°C and 200°C.

The XRD analysis revealed the presence of CuO and NiO phase only in the case of catalysts supported on SiO<sub>2</sub>. On the other hand, no XRD reflexes assigned to active phases are present in the diffraction patterns of catalysts supported on CeO<sub>2</sub> and ZrO<sub>2</sub>. This can result from the very small crystallites of CuO and NiO which are formed on the surface of CeO<sub>2</sub>, below the detection limits of XRD. Nevertheless, the presence of copper and nickel on the support was confirmed by XRF analysis, which showed that determined concentration values of both active phases closely corresponds with nominal values. In the H<sub>2</sub>-TPR profiles of bimetallic catalysts overlapping signals of CuO and NiO reduction are present. The position of these signal depends on the used supports and for all catalysts is below the reduction temperature of standard CuO and NiO. In the case of all catalysts, the conversion of furfuryl aldehyde increases with the temperature of the reaction, reaching 73% for Cu<sub>1.0</sub>Ni<sub>1.0</sub>/CeO<sub>2</sub> at 160 °C. The selectivity to furfuryl alcohol is the highest when reaction is performed at 120 °C (33% for Cu<sub>2.5</sub>Ni<sub>2.5</sub>/SiO<sub>2</sub>). The increase of temperature causes that selectivity to furfuryl alcohol drops and furan starts to dominate among detected products. This proves that decarbonylation pathway is favored over hydrogenation of furfuryl aldehyde. Additionally, 2-MF and propane was also detected which suggests that hydrogenolysis and ring opening reactions occur over synthesized catalyst too.

To sum up, our experiments shown that the type of the support used for the active metal phase influences activity of the studied catalysts and their selectivity to different reaction products. The most active catalysts were those in which the active phase was supported on CeO<sub>2</sub>, while the least active those in which the active phase was deposited on ZrO<sub>2</sub>.

# **Physics and Chemistry of Surfaces and Nanostructures**



# Mechanisms and kinetics of protein immobilization on solid/electrolyte surfaces: “Mechanisms of human serum albumin corona formation at polymer and silica microparticle carriers”

(*Malgorzata Nattich-Rak DSc, Marta Sadowska PhD, Monika Wasilewska PhD, Katarzyna Kusak MSc, Professor Zbigniew Adamczyk*)

Coronavirus attachment to abiotic surfaces is a crucial issue controlling their inactivation and removal by filtration, which can decrease the epidemics proliferation. However, because of limited availability of intact virus samples, no systematic experimental works were devoted to a thorough physicochemical analysis of this process. It is postulated in the work that reliable information about mechanisms of virus attachment to abiotic surfaces can be acquired using a nanoparticles with protein corona, which can well mimic physicochemical parameters of virions.

Therefore, the main objective of this work was to elucidate the mechanism of protein corona formation and deposition kinetics of the virus-like particles formed in this way at solid/electrolyte interfaces. Attention was focused on the on silicon dioxide/human serum albumin (HSA) conjugate, which exhibits pronounced advantages. Initially, reference measurements of albumin adsorption kinetics on bare and macrocation (PARG) silicon dioxide sensors were carried out using reflectometry and the atomic force microscopy (AFM).

Results of these experiments were used as reference data for the analysis of the adsorption mechanism of albumin on the silicon dioxide particles of the size 140 nm, which resulted in the formation of protein coronas (Fig. 1). The influence of ionic strength on the maximum coverage and the stability of the suspensions was determined in these experiments. It was confirmed that the electrostatic interactions between the protein molecules and the silica nanocarriers played a dominant role.

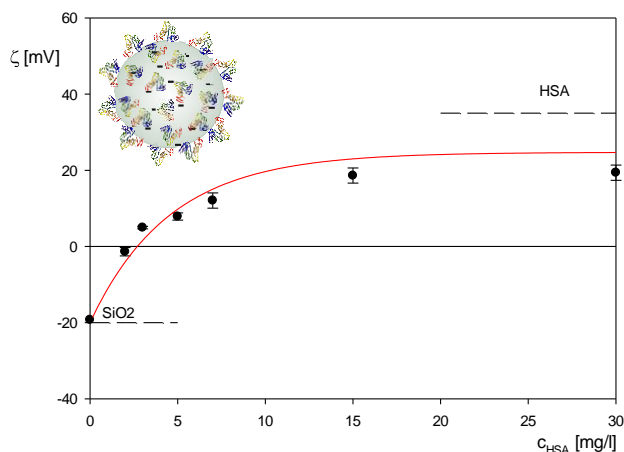


Fig. 1. Dependence of the zeta potential of silicon dioxide nanoparticles on the HSA concentration in the suspension: pH 4, 10 mM NaCl, particle concentration 500 mgL<sup>-1</sup>, (●) experimental results derived from the LDV measurements. The solid line shows the theoretical results calculated from the general electrokinetic model.

Concluding, the results acquired in this work showed that it was feasible to prepare stable polymer particles with albumin corona characterized by well-defined physicochemical characteristics. Such particles can be used as reference systems for predicting kinetics of intact virus deposition on abiotic surfaces and for their efficient removal by various filtration procedures.

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

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Fluorescent metal nanoclusters, synthesized on protein scaffolds, exhibit notable features such as a high Stokes shift, excellent photoluminescence, photostability, and low toxicity compared to quantum dots. Consequently, they find wide applications in genetic diagnostics, targeted drug delivery, catalysis, and chemical analysis. Numerous methods for preparing fluorescent nanoclusters of silver, gold, and copper using proteins like albumins (HAS, BSA),  $\gamma$ -globulins (IgG, IgM, IgA), transferrin, and lysozyme have been developed. However, understanding conformational changes and electrokinetic properties resulting from strongly alkaline reaction conditions and metal incorporation into protein structures remains unclear. Furthermore, self-assembly processes of metal nanoclusters at solid/liquid interfaces have not been documented in the literature. Addressing these gaps, the primary objective of this study was to investigate the deposition processes of fluorescent metal nanoclusters on the surfaces of colloidal carriers, including silica and polystyrene particles.

Three types of gold nanoclusters (AuNCs) were synthesized using a chemical reduction method with human serum albumin (HSA), bovine serum albumin (BSA), and hen egg white lysozyme (HEWL) as stabilizing agents under alkaline conditions. High-resolution transmission electron microscopy (HR-TEM) imaging revealed that the average size of the metal core was consistent at  $1.5 \pm 0.5$  nm, irrespective of the type of protein employed. However, the hydrodynamic diameter of the nanoclusters ranged between  $5.1 \pm 0.2$  nm and  $6.3 \pm 0.3$  nm for HEWL-AuNCs and HSA-AuNCs, respectively. The nanoclusters exhibited robust luminescence at a wavelength of 650 nm, with the intensity significantly decreasing under acidic conditions ( $\text{pH} < 3.5$ ). Dynamic light scattering technique (DLS) studies indicated that aggregation, manifested by an increase in hydrodynamic diameter, occurred within the pH range of 4.3 to 7.8 for all types of nanoclusters. Beyond this range, the nanoclusters remained stable at an ionic strength lower than 0.15 M. Additionally, a considerable influence of ionic strength and pH on the electrokinetic properties of the clusters was observed. The isoelectric points of the nanoclusters were identified within the pH range of 5.6-5.8 (for HSA-AuNCs and BSA-AuNCs), aligning well with the isoelectric points of the respective albumins. At a given pH, an increase in ionic strength led to a decrease in the zeta potential of AuNCs. For example, at pH 4.0, the zeta potential of HSA-AuNCs was  $80 \pm 2$  mV and  $15 \pm 3$  mV for ionic strengths of  $10^{-4}$  and 0.15 M, respectively.

Having AuNCs of well-defined physicochemical properties, the electrostatically driven self-assembly processes on the surfaces of sulfonate and amidine polystyrene particles of an average size  $800 \pm 15$  nm and  $820 \pm 23$  nm was investigated. The dependences of electrophoretic mobility and zeta potential of polystyrene particles on the concentration of AuNCs in the suspensions were determined for given conditions of pH and ionic strength.

The research conducted at pH 4.0 revealed that the zeta potential of microspheres increases with the concentration of HSA-AuNCs from  $-60 \pm 3$  mV to  $40 \pm 3$  mV. This indicated the formation of monolayers of nanoclusters on the surfaces of colloidal particles. The formed nanocluster layers remained stable at pH 4.0 and ionic strengths not exceeding  $10^{-2}$  M. Furthermore, through confocal microscopy imaging and spectrofluorimetric measurements, it was demonstrated that the deposited monolayers of nanoclusters retained their fluorescent properties. Thus, the achievement of fluorescent colloidal particles with sizes significantly larger than those of unbound nanoclusters was confirmed.

## **Physicochemical and mechanical properties of "bio" systems**

### **Physicochemical and mechanical properties of biosystems**

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

The aim of the task was to study systems defined as 'bio' in terms of their physicochemical (such as charge, shape and size distribution of molecules and particles as a function of environmental parameters, internal structure of selected molecules) and mechanical (such as Young's moduli, spatial packing structure) properties.

The statutory task complemented and developed the research carried out under the 2022 statutory task carried out by the members of the group 'Nano and micro scale systems'. In continuation of the 2022 statutory research, we worked further on the computer modelling of indentation performed with an atomic force microscope, We continued to focus our efforts on the modelling of colloidal probe experiments. The method for conducting simulation studies was the finite element method. During the year, we developed a model of the cell based on its interior being filled by a viscoelastic fluid, which is a better approximation than commonly used models that do not take into account energy dispersion due to frictional forces importantly, we performed modelling comparisons assuming constant internal pressure with a constant volume model. We also used a layer of so-called cortex as a bounding element for the cell from outside.

The proposed model is relatively easy to use (comparable to the Hertz function) to extract cell properties from a measurement, with additional consideration of the internal structure of the cell. We have also worked on the behaviour of macro ions depending on the parameters of the environment they are in. We have also worked on methods to analyse atomic force microscopy data, in particular to analyse the structure of fibrils formed from various proteins associated with neurodegenerative diseases (tau protein and amyloid beta)<sup>1</sup>. We have also conducted work on proteins and their structure in the context of their use in the food industry<sup>2</sup>.

[1] Nanoscale insights into the local structural rearrangements of amyloid- $\beta$  induced by bexarotene, Kamila Sofińska, Piotr Batys, Adrian Cernescu, Dhiman Ghosh, Katarzyna Skirlińska-Nosek, Jakub Barbasz, Sara Seweryn, Natalia Wilkosz, Roland Riek, Marek Szymoński, Ewelina Lipiec, *Nanoscale* 15 (35), 14606-14614

[2] The influence of thermodynamic qualities of a solvent on the physicochemical properties of lentil protein concentrate—Second virial coefficient study, Daniel Żmudziński, Urszula Goik, Paweł Ptaszek, Anna Ptaszek, Jakub Barbasz, Joanna Banaś, Dawid Lupa, *Food Chemistry* 434, 137329

## **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., Ewa Madej PhD Eng., Ewa Młyńczak PhD, Robert Socha PhD, Dorota Wilgocka-Ślęzak PhD Eng., Bohdana Blyzniuk MSc, Natalia Kwiatek MSc)*

Research on spintronic systems based on antiferromagnetic 3D metal oxides conducted in cooperation with AGH was continued. The properties of NiO(001) thin films in epitaxial NiO/MgO(d)/Cr and Fe/NiO/MgO(d)/Cr heterostructures on MgO(001) substrates were studied as a function of the thickness  $d$  of the MgO layer. X-ray magnetic linear dichroism measurements showed that with increasing  $d$ , NiO spins rotate from a direction in the film plane to the perpendicular direction. The modification of the magnetic anisotropy of the antiferromagnetic NiO layer, responsible for this process, was explained by the change in epitaxial strain at the NiO/MgO interface. It has also been shown that the exchange interactions of NiO with the ferromagnetic Fe layer can also be used to modify the magnetic anisotropy of the antiferromagnetic material.

Another collaboration with AGH concerned determining the influence of external proteins (endogenous factors) and tungsten oxide nanoparticles (exogenous factor) on the activity of the photosystem II (PSII), and in particular on the process of oxygen evolution. For this purpose, a new type of self-assembled hybrid system was created in a colloidal form, using semiconductor (n-type) nanoparticles (NPs) of tungsten oxide ( $\text{WO}_3$ ) and membranes isolated from spinach, the so-called PSII BBY. Such a hybrid system should have an ability to release oxygen similar to the efficiency of the natural PSII system, and at the same time it does not require additional charge carriers and is capable of operating in neutral water conditions. The photosynthetic activity of the PSII BBY- $\text{WO}_3$  NPs system was investigated using absorption spectroscopy, fluorescence methods and fast polarography. Atomic force microscopy and electron microscopy were used to investigate the influence of  $\text{WO}_3$  NPs on the organization of PSII BBY. It was found that the heterogeneity of PSII operation is the result of both the heterogeneity of the states of the manganese complex and the long-range interactions of the hydrogen network formed by proteins and water molecules. For the first time, a hybrid system without additional aqueous carriers was obtained, operating in a neutral aqueous environment, which exhibits activity comparable to the natural PSII system.

A newly undertaken topic was the study of ultra-thin Sn films on Pt(111) substrates. This system is interesting, among others, due to the improvement of catalytic properties compared to pure platinum, e.g. for the CO oxidation reaction and oxygen reduction reaction. The initial phases of Sn growth on Pt(111) were studied using a LEEM microscopy and the evolution of the emerging superstructures was observed with increasing coverage (initially  $\sqrt{3}\times\sqrt{3}(\text{R}30^\circ)$ , then  $c(4\times 2)$ ). The growth mode was also followed in the real space and a strong influence of the structure of Pt(111) atomic terraces on the organization of Sn atoms was noted.

As in previous years, our laboratory performed several dozen XPS analyses, the aim of which was to determine the chemical state and oxidation degree of catalytic and other functional materials.

As part of cooperation with the National Synchrotron Radiation Center Solaris, the group was involved in the maintenance of the instrumentation infrastructure and measurements on two research lines: DEMETER and PIRX. As part of this activity, the group members took part in the implementation of several research projects, both their own and those coming from external users from Poland and abroad.



## Computer-aided design of hybrid nanoparticles for cancer theranostics applications

*(Professor Tomasz Pańczyk, Professor Wojciech Płaziński, Agnieszka Brzyska PhD, Paweł Wolski PhD)*

The properties of nanoparticles can be altered through surface modification using various ligands, such as polymers with diverse internal structures and chemistry. This enables the fine-tuning of particle properties across a broad spectrum, thereby expanding their potential applications in various fields. In this study, we explored the covalent functionalization of carbon quantum dots (CQDs), a novel type of carbon nanomaterials, with fourth-generation poly(amidoamine) (PAMAM) dendrimers using all-atom molecular dynamics simulations.

Specifically, we investigated the impact of the grafting technique (grafting-from and grafting-to) and the oxidation level of CQDs on the functionalization. Molecular simulations were conducted on four model systems of CQD-G4 PAMAM hybrid nanoparticles. Two CQD models, with 10% and 60% edge coverage with oxygen functional groups (carboxyl and hydroxyl), underwent grafting according to the grafting-from and grafting-to methods, with two G4-PAMAM dendrimers attached to the opposite side of the nanoparticle.

The obtained results revealed that the extent and nature of dendrimer wrapping onto the CQD surface depend on both the grafting approach and the oxidation levels of the nanoparticle. In the grafting-from approach, an increase in the oxidation level of the CQD resulted in a reduction in the surface area covered by dendrimers from 70% to 30%. In contrast, in the grafting-to case, dendrimers covered approximately 50% of the nanoparticle surface, regardless of the oxidation level of CQDs. This suggests that the grafting-to approach may be more effective in achieving a uniform surface coverage of the CQD, irrespective of the number of oxygen functional groups on the surface.

Furthermore, in reduced cases, grafting-from dendrimers assumed a flat, disk-like conformation. Conversely, for the grafting-to approach, an increase in the number of dendrimer branches led to heightened steric repulsion among them, resulting in more expanded conformations with a substantial number of monomers rising above the CQD surface. For higher oxidation levels of CQDs, the structure of adsorbed dendrimers became more compact in both grafting approaches. This characteristic structural feature was attributed to the increasing electrostatic interactions between the protonated primary amine groups of dendrimers and the carboxyl groups of the CQD, leading to the dense packing of terminal groups close to the CQD surface.

The calculated pair-interaction energies and the number of H-bonds demonstrated that the higher oxidation level of the CQD provides stronger interfacial interactions between dendrimers and CQD, regardless of the grafting approach. Additionally, grafting-from dendrimers exhibited stronger interactions with CQD compared to grafting-to cases. Consequently, the intramolecular structure of the CQD nanoparticle was better preserved in the grafting-from approach. Moreover, we observed that the redistribution in the position of dendrimer terminal groups induced by the higher oxidation level of CQD decreased the binding activity of dendrimers with other molecules (water in this case). This suggests that the interparticle interactions of the CQD-G4 PAMAM composite can be controlled by tuning the polymer–substrate interaction strength. Overall, the conducted research demonstrated that the surface chemistry of CQDs and the grafting approach of dendrimers play a crucial role in governing the structure/property relationships of CQD-G4 PAMAM hybrid materials.

## **Determination of mechanisms of synthesis, structure, and transport parameters of supported colloidal particle multilayers**

*(Associate professor Paweł Weroński)*

The surface roughness of a particle monolayer is one of its fundamental characteristics, conventionally described in terms of power spectral density (PSD). We derived a general equation for this function, valid also for systems of a finite size. We demonstrated similarity of the equation to those applied in the theory of scattering. Specifically, the PSD is a linear function of the squared form factor and static structure factor. The form factor is a Fourier transform of the particle height profile. The structure factor is equal to the product of particle number density and Fourier transformed pair-correlation function calculated in the finite area, increased by one. The relationship suggests that roughness measurements of particle monolayers can be considered as a type of spectroscopy, in a wider sense of the term.

We also demonstrated that our results can be generalized to virtually any monolayer image. Therefore, our research provides a framework for a quantitative, cheap, and easy method, based on statistical analysis of the digital monolayer image, for determining the surface roughness and particle pair-correlation function. The approach can be an attractive alternative to standard, expensive, experimental methods such as GISANS or GISAXS. The PSD provides also quantitative information on key parameters of the system: the particle radius, number density, and dimensions of analyzed area. Therefore, the theoretical model provides also a general framework for an automatic, potentially real-time parametrization of particle monolayers. Unlike in the methods currently used in image analysis, relying usually on individual particle identification, we do not have to localize each of the particles here. Instead, we just need to calculate the discrete Fourier transform of the monolayer image and to least-squares fit the monolayer parameters.

We showed that our analytical results are consistent with numerical ones derived for a system with parameters corresponding to typical experimental conditions. We also demonstrated a practical application of our approach for spectral analysis of a monolayer image found in the literature.

## Theranostic nanocarriers of anticancer drugs

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

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 2023, we were focused on the analysis of theranostic nanocarriers for targeted anticancer therapy. The theranostic nanocarriers of anticancer drugs were formed by the sequential adsorption of charged nanoobjects method (called layer-by-layer). The following anticancer drugs: SN-38, salinomycin and docetaxel were selected for encapsulation into multilayered core@shell nanocarriers formed with poly-L-lysine (PLL), gadolinium labeled poly-L-lysine (PLL-Gd), poly-L-glutamic acid (PGA) and pegylated-PGA (PGA-g-PEG). The size of prepared nanocarriers ranged between 80-200 nm. Figure 1 presents cryo-SEM image of analyzed theranostic polyelectrolyte nanocarriers. The properties of developed nanocarriers were optimized for passive targeting based on the EPR effect. The effects of encapsulated anticancer drugs: Salinomycin and docetaxel on the viability of the 4T1 mammary carcinoma and CT26-CEA murine colorectal carcinoma cell lines were examined. Encapsulated drugs show high anticancer activity similar to the tested free drug.

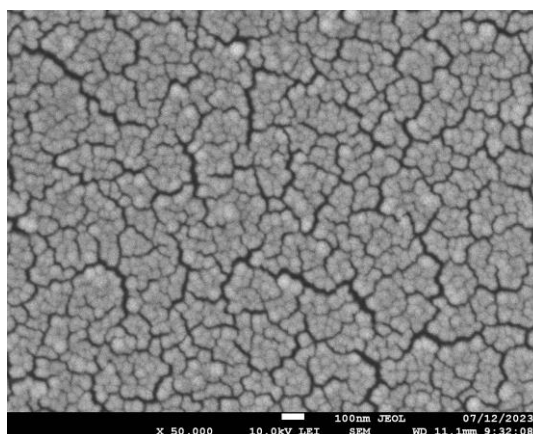


Figure 1. The Cryo-SEM image of polyelectrolyte nanocarriers.

## Kinetics of coalescence in water-in-water emulsions

(Associate professor Jan Zawala, Andrzej Baliś PhD Eng., Dominik Kosior PhD, Georgi Gochev DSc, Agata Wiertel-Pochopień PhD Eng., Mariusz Borkowski MSc, Łukasz Witkowski MSc, Klaudia Zaręba MSc)

The aim of the task is to determine the kinetics of coalescence of drops formed in two-component polymer systems (Aqueous Two-Phase Systems - ATPS), which, in specific proportions, undergo spontaneous separation. ATPS are very interesting systems of two immiscible aqueous solutions in thermodynamic equilibrium. Such systems are very interesting from a practical point of view (ATPS can be used in the processes of extraction, separation, purification and enrichment of cell organelles, proteins, carbon nanotubes and metal ions as well as for applications in controlled drug delivery), and their greatest advantage is the high level of biocompatibility (no organic phase as in conventional oil-water O/W emulsions). The basic problem in the use of ATPS and capsules made from them for targeted drug delivery is the low degree of dispersion stability, caused by extremely low interfacial tension. The research work planned in this task is aimed at searching for effective stabilizers for ATPS systems that would significantly expand their scope of application and functionality.

During the reporting period, stability measurements were carried out on ATPS emulsions, which were obtained by mixing polyethylene glycol (PEG) and dextran (DEX) in appropriate proportions. Polymers with different molecular weights ( $M_w$ ) were selected for testing. It was found that the greater the difference in the  $M_w$  of both polymers, the greater the stability of the obtained emulsion. However, this stability was not satisfactory (see Fig. 1A), which was caused by the lack of an appropriate stabilizer that could prevent the coalescence phenomenon – the process responsible for delamination of the system. Nanogel particles of BLG protein (beta-lactoglobulin), which were obtained during optimized synthesis, were selected as the stabilizer. Thanks to precise pH control during syntheses, monodisperse particles were obtained, the sizes of which ( $d_{\text{blg-gels}}$ ) were in the range of 30-130 nm. The addition of a specific concentration of nanoparticles to the solution of one of the polymers before the emulsification process resulted in a spectacular increase in emulsion stability, which was greatest for particles with sizes of 50-80 nm. In order to investigate the mechanism of this phenomenon, measurements of interfacial tensions ( $\gamma$ ) of polymer systems with the addition of nanoparticles were carried out using the spinning drop method (see Fig. 1B). The measurements showed that there is no correlation between the degree of reduction in interfacial tension caused by a specific size of nanoparticles and the stability of the emulsion, so the stabilization mechanism is more complex in this case. Subsequent planned studies (including observation of the formation of an adsorption layer in the interfacial area of drops using a confocal microscope) will aim to explain the impact of the stabilizer size on the properties of the tested ATPS.

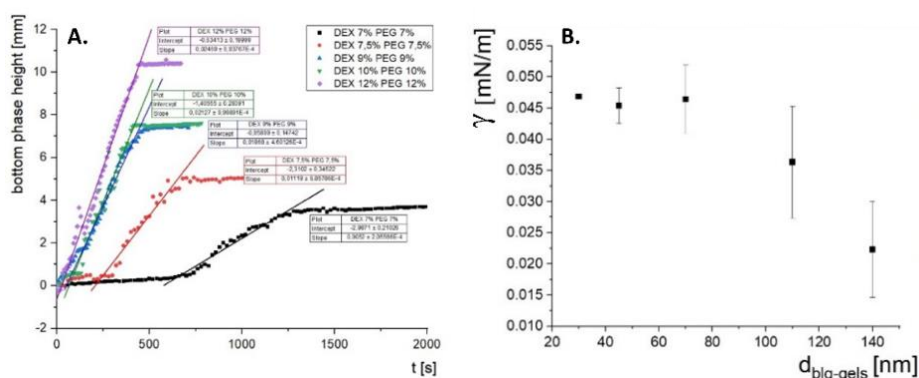


Fig. 1. Graphs showing (A): kinetics of separation of ATPS type emulsions depending on the difference in the molecular weights of mixed polymers and (B): values of interfacial tensions obtained by the spinning drop method, as a function of size of the stabilizer's nanoparticles.

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

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

In the frame of the statutory task, we have investigated the surface properties of emulsion systems containing polysaccharides: chitosan-polycation and furcelleran-polyanion in the aqueous phase and plant essential oils (oregano, thyme, rosemary and lemongrass) used as the organic phase. The high application potential of plant essential oils is a consequence of their antibacterial and antioxidant properties. We have obtained similar surface tension values at the water/essential oil as well as at an aqueous solution of polysaccharides/essential oil interfaces, and it was equal to  $15 \pm 1$  mN/m. Thus, the nonionic surfactant Tween 80 was applied in order to reduce surface tension and facilitate the emulsification process. However, the addition of surfactant significantly decreased the surface tension in the case of the system with rosemary and lemongrass essential oils. We have not observed a significant change in the surface tension value at the interface with oregano and thyme oil (Fig.1).

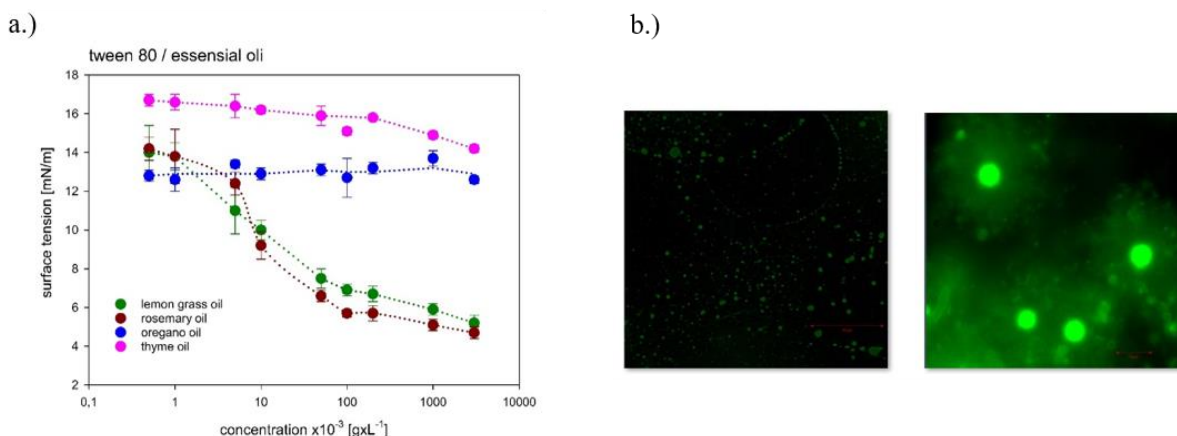


Fig.1.a. The dependence of the equilibrium surface tension on the concentration of Tween 80 at aqueous surfactant solution/essential oil interface. b. The confocal microscopy images of emulsions with 1000 ppm of chitosan in 1% acetic acid in the presence of Tween 80 and oregano essential oil as the organic phase.

The collected IR spectra of essential oils confirmed the significant differences in the molecular structures of the compounds they contained (including the presence of OH groups in oregano and thyme oil). In the case of the chitosan/oregano system the confocal microscope images revealed the presence of characteristic structures near the interface (Fig.1b). The research will be continued to explain the mechanism of the formation of such complexes. Moreover, we carried out additional studies on the surface activity of aqueous solution of chitosan modified with hydrophobic hydrocarbon chains of various lengths (C8, C12, C16) and we examined how the length of the hydrocarbon chain and type of linker with polysaccharides skeleton, either through an amide or ester bond, affected the wettability and surface energy of the polyelectrolyte multilayer containing the modified polysaccharide.

## New electrolyte materials for solid oxide fuel cells with reduced operating temperature

(Michał Mosiałek DSc, Grzegorz Mordarski PhD Eng., Amir Sultan MSc Eng.)

The Electrochemistry Group in the Soft Matter Nanostructures Group conducts research on new materials for solid oxide fuel cells. The high sintering temperature of the mixed oxides  $\text{BaCe}_{1-x-y}\text{Zr}_y\text{Y}_{0.1}\text{O}_3$  generates high costs of their preparation as well as makes it difficult to obtain porous materials for electrodes in the form of composites with mixed oxides showing ion-electronic conductivity with a much lower sintering temperature.

The aim of the research conducted in 2023 was to produce electrolyte materials based on  $\text{BaCe}_{1-x-y}\text{Zr}_y\text{Y}_{0.1}\text{O}_3$  with a reduced sintering temperature and to test their ionic conductivity using electrochemical methods. Based on the analysis of the literature, we found that small additions of other metal oxides, especially copper, can significantly reduce the sintering temperature. The obtained electrolyte materials  $\text{BaCe}_{1-x-y-t}\text{Zr}_y\text{M}_t\text{Y}_{0.1}\text{O}_3$  ( $\text{M} = \text{Ni}$ ) were characterized using available physicochemical methods, e.g. XRD, SEM-EDS, TG-DSC and electrochemical tests.

$\text{BaCe}_{0.5}\text{Zr}_{0.3}\text{Y}_{0.2}\text{O}_3$ -delta (BCZY), considered one of the most promising proton-conducting electrolytes, poses difficulties due to the high sintering temperature. To solve this drawback, the  $\text{BaCe}_{0.56}\text{Zr}_{0.2}\text{Ni}_{0.04}\text{Y}_{0.2}\text{O}_3$ -delta (BCZY) electrolyte doped at the B position was produced. The effect of Ni addition on the electrical properties and sintering parameters was investigated. By adding Ni in the B position of BCZY, a significant drop in the sintering temperature was achieved, i.e. from 1600 degrees C (for BCZY) to 1300 degrees C (for BCZMY). For the BCZMY electrolyte in a humid nitrogen environment, a conductivity of  $0.05 \text{ S cm}^{-1}$  at a temperature of 700 degrees C was observed. Moreover,  $\text{SrFe}_{0.8}\text{Mo}_{0.2}\text{O}_3$ -delta (SFM) was used as a symmetrical electrode material due to its high ionic and electronic conductivity. The SFM electrode was synthesized in the combustion process and showed appropriate chemical and thermal compatibility with BCZY and BCZMY electrolytes up to 1100 degrees C. The single-phase cubic structure along with the surface morphology of symmetrical cells based on the proposed electrolytes was confirmed using X-ray diffraction and scanning electron microscopy. Electrochemical impedance spectroscopy was performed to determine the area polarization resistance (ASR). The lowest ASR values of 0.037, 0.081 and  $0.327 \text{ } \Omega \text{ cm}^{-2}$  at temperatures of 800, 700 and 600 degrees C in humidified hydrogen were obtained for the SFM-BCZNY composite electrode. The symmetrical cell achieved a peak power density of  $254 \text{ mW cm}^{-2}$  (cell configuration; SFM-BCZMY | BCZMY | SFM-BCZMY) at a temperature of 800 degrees C. Fuel cell devices in the configuration shown demonstrated long-term stability for 100 h at 700 degrees C under load  $120 \text{ mA cm}^{-2}$ . Considering the presented results, BCZMY is a promising proton electrolyte for IT-SOFC.

## **Influence of severe plastic deformation of Mg on the properties of phosphate layers**

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

In 2023, we have focused on three types of conversion coatings: phosphate (CaP), polylactide-poly lactide (PLA) and a combined CaP-PLA coating on the surface of WE43 magnesium alloy. Scanning electron microscopy, X-ray diffraction, X-ray photoelectron spectroscopy, and infrared spectroscopy were used to characterize the surface and interface morphology as well as the composition of the coatings before and after corrosion experiments. The protective properties of the obtained coatings were tested by potentiodynamic polarization and immersion tests in Hanks' solution. The results allowed us to propose a mechanism for coating deposition and corrosion degradation.

In long-term corrosion experiments, the CaP layer did not undergo significant structural, morphological and phase changes. However, due to local coating defects (cracks, pores), several local corrosion spots were observed, which is consistent with the increase in the pH of the Hanks' solution.

Deposition of a PLA layer on the surface of the WE43 alloy creates a defect-free polymer coating with a thickness of approximately 20  $\mu\text{m}$ . The corrosion rate of WE43 alloy treated in Hanks' solution decreased by 100 times. However, due to high water permeability and low adhesion to the substrate, this coating does not provide anti-corrosion protection of the substrate in the long term.

The two-component CaP-PLA coating provided improved corrosion resistance in Hanks solution for 14 days compared to untreated WE43 alloy. After long-term corrosion tests in Hanks' solution, the two-layer coating did not show any visible structural changes, and the pH of the solution practically did not change over time, which proves the high protective properties of the CaP-PLA coating.

In parallel, research was carried out on the influence of severe deformation on the properties of the AZ91 T6 magnesium alloy. Electrochemical tests of the AZ91 alloy subjected to surface mechanical abrasive treatment (SMAT) were carried out in 0.05 M NaCl for 24 hours.

The results of electrochemical impedance spectroscopy showed that for the mechanically processed AZ91 alloy, the polarization resistance values increased with the immersion time in the NaCl solution and after 10 hours they reached values much higher than those measured for undeformed reference samples of the AZ91 alloy. After more than 20 hours of immersion in NaCl solution, the determined resistance values for samples subjected to SMAT are similar to those obtained for reference samples.

Long-term (168 h) hydrogen evolution tests were performed in a 3.5% NaCl solution for SMAT-treated AZ91 samples and reference ones. Hydrogen evolution tests showed that for the deformed samples, the hydrogen evolution was slightly lower than for the reference samples.

The SMAT process affects the behavior of the tested magnesium alloy during corrosion, making it more reactive, which in turn accelerates the formation of a layer of oxidation products on its surface during immersion in NaCl solutions. This, in turn, leads to an improvement in its corrosion resistance in the first hours of immersion.

## **Physicochemical properties of functional nanocarriers based on dendrimers and proteins**

*(Professor Barbara Jachimska, Agnieszka Kamińska PhD, Magdalena Szota, Łukasz Luśtyk)*

Studies on the development of Parkinson's disease have shown that it is characterized by the presence of Lewy bodies containing mainly  $\alpha$ -synuclein. In Lewy bodies,  $\alpha$ -synuclein is misfolded and can cause neuronal death. Understanding how soluble proteins create amyloid forms and trigger the cellular toxicity pathway is the basis for developing effective therapies for Alzheimer's, Parkinson's and Creutzfeldt-Jakob diseases. It should be emphasized that the control of changes in the structure of amyloid proteins at the molecular level, and thus the identification of key elements that initiate the cascade of changes ultimately leading to the formation of chiral superstructures, is still insufficiently known. Recently, it was discovered that proteins responsible for neurodegenerative diseases interact electrostatically with negatively charged glycolipids located on the surface of nerve cells. Since electrostatic interactions seem to be one of the key factors contributing to the aggregation of protein systems on the surface of the cell membrane, the research was carried out by controlling the electric potential and surface charge density. Using advanced analytical methods, the transformation of the monomeric form into protein aggregates in solution and on the interfacial surface was monitored in situ, imitating the conditions prevailing during protein aggregation in cells and tissues. The use of a coordinated methodological approach, including the use of a quartz microbalance (QCM-D), surface plasmon resonance (MP-SPR), and infrared spectroscopy (IR), allows us to understand the course of the biochemical process at the submolecular level. Circular dichroism (CD) and infrared (IR) spectra confirmed that the interaction of the protein with the surface induces significant conformational changes. The direction and intensity of structural changes depend on many factors, including the pH of the environment in which the research was carried out.



## **Physics and Chemistry in Cultural Heritage Protection**



## **Quantitative assessment of the threat to historic objects caused by environmental conditions in their surroundings**

*(Marcin Strojecki PhD, Sergii Antropov PhD, Professor Łukasz Bratasz)*

The research activity conducted within the task was aimed at the development of a comprehensive model of indoor particle deposition onto surfaces of historic interiors. The model takes into account the most important deposition processes observed in historic buildings as Brownian and turbulent diffusion, gravitational settling, turbophoresis, and thermophoresis. There are several novel and significant aspects of this work:

- the model is expressed as a function of important parameters characterizing historic interiors as the friction velocity - capturing the effect of the indoor airflow intensity, the difference between the temperature of the air and the surface, and surface roughness,
- all formulae used in the model are parametrized by the air temperature and particle size and density,
- new forms of the air turbulent viscosity and the thermophoretic term were proposed to account for an important mechanism of surface soiling driven by frequent large temperature differences between indoor air and surfaces in historic buildings. The form adopted allowed the temperature gradients to be calculated down to low distances from the surfaces and showed insignificant dependence of the temperature gradient on the diameter of particles, which yielded a meaningful physical description of the process.

The predictions of the model developed agreed with the outcome of the previous models, in turn correctly interpreting the experimental data. The model was used in simulating the total deposition velocity in a small-size church taken as an example of a historic building with heating operating in the cold period. In the simulations, two extreme values of the friction velocity assumed for historic buildings - 1 and 6 cm/s - as well as two extreme degrees of surface roughness - smooth wallpaper and rough wall plaster - were used, whereas the temperature difference between the interior surfaces and air was varied between  $-3$  and  $2$  °C.

The model adequately predicts the deposition processes observed in historic buildings and proves to be able to map magnitudes of deposition velocities for specific surface orientations as functions of air-surface temperature gradient, surface roughness and airflow intensity reflected in the friction velocity. One of the most important achievements and practical outcomes is an indication that the common conviction that an increase in the temperature of the surface can significantly lower the deposition rate is not true. The model significantly deepens the basic knowledge about one of the key environmental risks to historic interiors, i.e. the rate at which architectural surfaces and valuable interior furnishings become visibly soiled. It also enables the interpretation of an increasing number of research results of the indoor environment in various historical buildings. Therefore, the study can be used to develop new methods of protecting objects against soiling by conservators and object owners interested in assessing and mitigating environmental risk to various types of historic interiors.



**"Sonata Bis" Research Projects  
of the National Science Centre**



# 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: Associate professor Jan Zawala)

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, an experimental system was designed and constructed that allows direct measurement of the thickness of foam and wetting films using the interferometric method in dynamic conditions (Dynamic Fluid-Film Interferometry - DFI). For this purpose, purchased optical and electronic components were integrated into a compact device, the individual elements of which were synchronized using appropriate software with a user-friendly graphical interface. The principle of operation of the device is to create an air bubble of a controlled volume (diameter) on a steel needle under the interface (solution/air, in the case of testing the stability of wetting films or solution/solid, in the case of wetting films) and automatically pressing the bubble to the interfaces with a specific force causing the film, i.e. a thin layer of liquid separating the contacting surfaces, to drain. Thanks to the use of a pressure sensor, it is possible to control the increase in pressure inside the bubble and thus control the pressure force, which affects the drainage rate, determining the deformation of the bubble, i.e. the radius of the resulting liquid film. In this way, it is possible to observe the created film and directly measure its thickness, and thus determine the impact of physicochemical conditions on its stability. This is possible thanks to the use of an optical system equipped with an appropriate lens, a filter that transmits only three specific wavelengths, a digital camera and LED lighting. Tests of the created device confirmed its enormous potential. Thanks to the developed experimental system, it will be possible to conduct research aimed not only at qualitative assessment of the stability of liquid films, but also at examining the mechanism of their rupture and comparing the kinetics of drainage under various conditions, depending on the planned change in external factors. A schematic illustration of the device geometry and the obtained photos of a single liquid (wetting) film illustrating its drainage process (changes in interference pattern) are shown in Fig. 1.

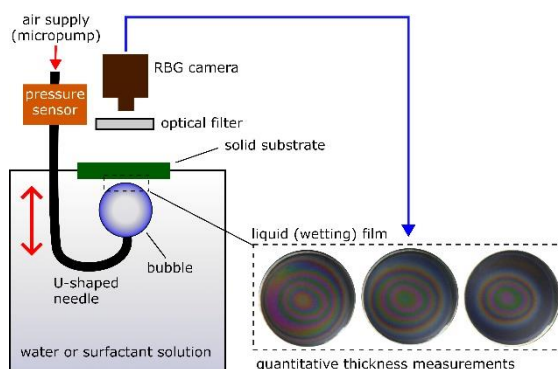


Fig. 1. Schematic illustration of the developed automatic system for observing and measuring the thickness of single liquid films (the presented photos of the films concern the wetting film formed between the bubble and the glass surface in pure water).

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



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

The TopoTin project was started on March 15, 2023, so at the end of 2023 we have completed approximately 9 months of project realization. The project is being realized in accordance with the assumed plan, we are in the process of carrying out the tasks planned for the first year of the project. As part of the project, we adapted the preparation chamber in an ultra-high vacuum system to perform the deposition of  $\text{Fe}_x\text{Sn}_y$  layers using the molecular beam epitaxy method. In December, we also launched an atomic force microscope with the ability to measure magnetic forces in an external magnetic field, purchased from the project funds.

The tasks planned for the project in the first year of its implementation were:

(A1) Development of growth protocols for the growth of Sn,  $\text{Fe}_3\text{Sn}$ ,  $\text{FeSn}$  and  $\text{Fe}_3\text{Sn}_2$  layers on conductive substrates. Here, so far we have focused on the growth of ultrathin Sn and  $\text{Fe}_3\text{Sn}$  layers on a metallic Pt(111) substrate. The deposition of layers was carried out using the molecular beam epitaxy method, and growth studies were initially carried out using a standard low-energy electron diffraction (LEED) instrument, and then using low-energy electron microscopy (LEEM), both in real space and in reciprocal space. Additionally, we examined the growth of  $\text{Fe}_3\text{Sn}_2$  layers on a substrate made of doped InSb(111), which has a metallic character. We examined the stoichiometry of the obtained samples using photoelectron spectroscopy (XPS) and their crystal structure using X-ray diffraction (XRD). We have also started the realization of task (A4), which was originally planned for the last year of the project, i.e. growth of selected structures on insulating substrates. Here, we used MgO(111) substrate to grow the  $\text{Fe}_3\text{Sn}_2$  layer.

(B1) Measurements of the magnetic properties of  $\text{FeSn}$  and  $\text{Fe}_3\text{Sn}_2$  layers. To accomplish this task, we performed measurements using the magneto-optical Kerr effect (MOKE), conversion electron spectroscopy (CEMS) and photoemission microscopy (PEEM, a working microscope at the Solaris synchrotron on the DEMETER line) on  $\text{Fe}_3\text{Sn}_2$  layers deposited on InSb(111) and MgO(111). We confirmed the existence of ferromagnetic order in the obtained layers at room temperature and the occurrence of full remanence for samples deposited on InSb(111).

(C1) Measurements of the electronic band structure of Sn,  $\text{Fe}_3\text{Sn}$ ,  $\text{FeSn}$  and  $\text{Fe}_3\text{Sn}_2$  layers. To accomplish this task, we carried out measurements using angle-resolved spectroscopy (ARPES) at the Phelix line at the Solaris synchrotron. So far, we have carried out measurements for Sn and  $\text{Fe}_3\text{Sn}_2$  layers deposited on InSb(111).

(C') Theoretical calculations of the band structure using the DFT method. This task is carried out in international cooperation with Dr. Gustav Bihlmayer and the Peter Grünberg Institute PGI-1. So far, calculations have been made of the electronic structure of Fe and the Sn layer deposited on Pt(111).



**"Sonata" Research Projects  
of the National Science Centre**



## 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.)

Extensive experimental characterization, combined with molecular simulations, was used to assess the impact of pH on the assembly mechanism and properties of poly(L-lysine) (PLL) and poly(L-glutamic acid) (PGA) complexes. A combination of dynamic light scattering (DLS) and laser Doppler velocimetry (LDV) was utilized to determine the complexation, charge state, and other physical characteristics of the complexes, isothermal titration calorimetry (ITC) was used to examine the complexation thermodynamics, and circular dichroism (CD) was used to extract the polypeptides' secondary structure. For enhanced analysis and interpretation of the data, analytical ultracentrifugation (AUC) was used to define the precise molecular weights of the peptides. Molecular dynamics (MD) simulations revealed the associated intra- and intermolecular binding changes in terms of intrinsic vs. extrinsic charge compensation, the role of hydrogen bonding, and secondary structure changes, aiding in the interpretation of the experimental data. The obtained data was combined to reveal the pH dependency of PLL/PGA complexation and the associated molecular level mechanisms.

Overall, the results showed that peptide effective charge is the most important factor in complexation of PLL and PGA. The complexation took place in the pH range of 4 to 10, which corresponds to the case of one of the polypeptides being at least partially charged. The complexed peptides adopted elongated assembly shapes, with molar fractions of the peptides governing assembly charge. Less intuitive are the secondary structure changes (see Figure 1) that occur via a combination of pH changes and complexation process itself, as supported by the CD results and MD simulations. Here, it was concluded that the secondary structures of the PLL/PGA systems were dictated by the polypeptide charge state. In the pH range of 4 to 10, where polypeptides were at least partially charged, clear changes in the secondary structures were observed upon complexation. The pH-dependent variation was also observed in the ITC measurements, indicating a multi-step association process between PLL and PGA. In addition, the trends with respect to complexation seem to be asymmetric with regards to pH, which was further explained, via MD simulations, by the differences in hydrogen bonding between the PGA and PLL. Moreover, it was shown that the increase in intermolecular hydrogen bonding between the polypeptides correlates well with  $\beta$ -sheet formation.

We provided the systematic physicochemical characterization of the pH dependency of PLL and PGA in solution and their complexes, which shows the dominant role of charge interactions in complexation and the systematic tunability by secondary structure changes, as well as the multistep character of the thermodynamics of assembly. This work provides not only guidelines for designing PLL/PGA complexes but also more generally polypeptide materials.

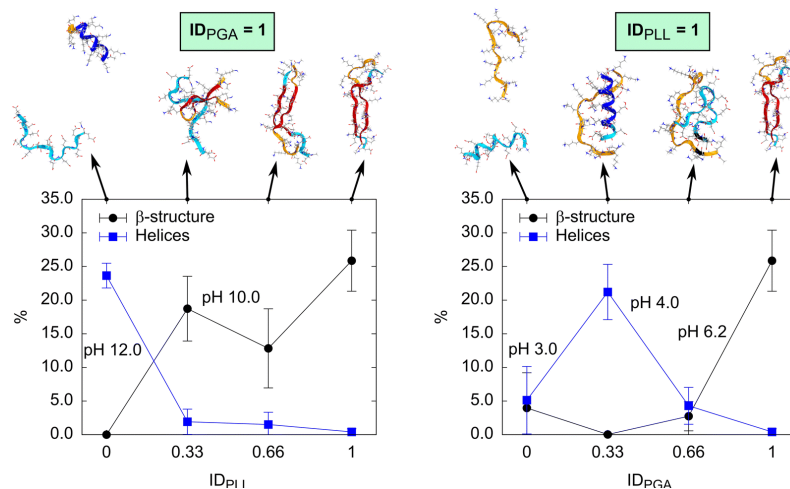


Figure 1. Secondary structure content (%) of helical and  $\beta$ -structures, and representative snapshots (on top) from the MD simulations at various PLL and PGA ionisation degrees (ID).

## 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, there has been a significant increase in interest in the use of inks based on metal nanoparticles (NPs) for the production of conductive tracks for "flexible" printed electronics. The process of fabrication of such conductive nanomaterials usually includes two stages: their deposition or printing on a suitable substrate and then sintering to transform the non-conductive ink into metallic, conductive structures. The most frequently used method for obtaining conductive tracks based on metal NPs has been thermal sintering so far, which is usually carried out at high temperatures. In order to avoid the destruction of heat-sensitive substrates used in the production of conductive flexible circuits and devices, alternative sintering methods performed at lower temperatures needed to be used. Silver nanoparticles have been the most often used so far for producing of conductive nanomaterials. However, due to their high cost and limited mechanical properties, other components are recommended. One of the candidates is nickel, which has good conductive and mechanical properties and is also much cheaper than silver. The problem is the instability of its nanoparticles regarding the oxidation process. Therefore, it was proposed to cover the nickel core (Ni NPs) with a silver protective coating ensuring good resistance to oxidation, and creating core-shell structures (Ni-Ag NPs).

The work carried out in 2023 focused on developing new methodologies for the fabrication of printed conductive coatings based on Ni-Ag NPs by using a low-temperature sintering process. Therefore, the effect of the wavelength as well as the time of UV-Vis irradiation on the conductive properties of such coatings were investigated. The obtained results suggest that the UV-Vis method is suitable for the obtaining of conductive coatings based on Ni-Ag NPs. The lowest value of resistivity  $\sim 24 \mu\Omega\cdot\text{cm}$  was obtained after 90 minutes of UV-Vis irradiation at a wavelength of 350-400 nm (Fig. 1). The calculated conductivity of such coatings corresponds to 29-30% of the conductivity of bulk nickel. The obtained results will contribute to significant progress and development in the field of nanotechnology by developing a new methodology for producing printed conductive materials on flexible substrates. Moreover, in the performed research, low-cost inks were used, which may be important from an economic and industrial point of view due to reducing the costs of the production process of electronic circuits and devices.

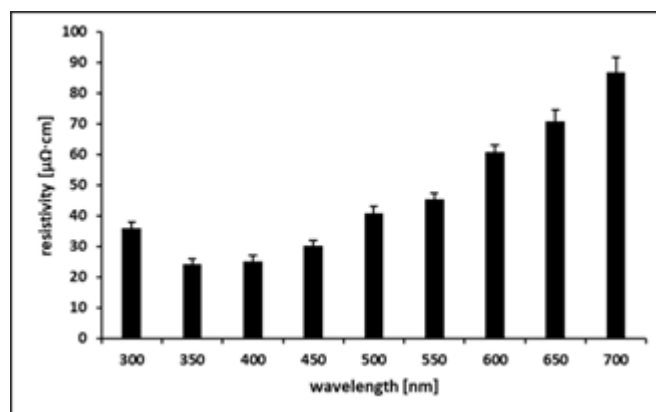


Fig. 1. The dependence of the resistivity of coatings based on Ni-Ag NPs on the wavelength of UV-Vis irradiation.

## **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 main goal of the research in the period in question (09-12/2023) was to apply a layer (or layers) of GO on the gold surface in a repeatable manner. The physicochemical characterization of the GO suspension was also carried out, including determining the size, zeta potential value and the functional groups present. QCM crystals whose surface is covered with gold and a gold foil with a thickness of ~30 nm applied to SPR glasses were used as model surfaces for deposition of graphene oxide. The deposited GO layer (or layers) should be characterized by high surface coverage, high stability and homogeneity. The number of GO layers should also be controlled. Several methods have been used to deposit GO on the gold surface, including: using "spin coater", "air brushing", immersion method and GO adsorption method on gold surfaces covered with thiols. Currently, work is aimed at optimizing GO deposition in a controlled manner and repeatable. The coverage of the gold surface with a GO layer (or layers) as well as its thickness and uniformity will be determined.



**"Opus" Research Projects  
of the National Science Centre**





## 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 goal of the project was to develop an efficient method for creating stable polyelectrolyte films that enable controlled attachment and release of fibroblast growth factors (FGFs), which may find application in the treatment of lifestyle diseases.

In the past year, the physicochemical characteristics of modified chitosan (MCHIT) were determined using molecular dynamics (MD) modeling and various experimental techniques. The research aimed to define the physicochemical properties of polyelectrolyte films based on MCHIT and carrageenan (K). The contour length of the MCHIT molecule and its equivalent chain diameter were determined. Interaction energies between MCHIT and K chains indicated a preference for a parallel orientation of the chains during the multilayer formation compared to a crossed orientation. It was demonstrated that MCHIT molecules possess a high positive electrokinetic charge over a wide pH range (up to pH 10), ensuring the stability of the polysaccharide solutions. It was confirmed that the polyelectrolyte film terminated by a K layer is significantly more stable than the film terminated by MCHIT. It was quantitatively interpreted in terms of the ion pair approach. It is worth emphasizing that the obtained results have practical significance as they enable the development of effective procedures for creating biocompatible polyelectrolyte films, which can be used as a platform for protein immobilization, in the creation of capsules in various drug delivery systems, and in antibacterial food packaging.

In the next stage MD and advanced research techniques, such as multi-angle dynamic light scattering, streaming potential, optical waveguide light spectroscopy, quartz crystal microbalance with dissipation, and atomic force microscopy, were applied to determine the basic physicochemical parameters of fibroblast growth factor 21 (FGF21). For the first time, the fundamental physicochemical parameters of the protein were determined, including the molecule's shape, cross-sectional area, the dependence of the nominal charge on pH, and the isoelectric point (IEP) at 5.3.

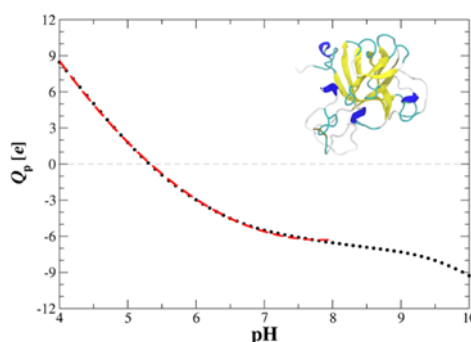


Fig. 1 Nominal charge ( $Q_p$ ) of the FGF21 molecule vs. pH calculated using the PROPKA 3.0 algorithm (dotted curve). The dashed red line represents the charge calculated from the polynomial fitting function. In the top right corner - the structure of FGF21 obtained from MD calculations.

It has been demonstrated that FGF21 irreversibly adsorbs onto the polycation layer (poly(diallyldimethylammonium chloride)), forming layers with controlled coverage up to  $0.8 \text{ mg m}^{-2}$ , while its adsorption on silica is less efficient. The viability of two cell lines, CHO-K1 and L-929, was determined on both uncovered and bilayered polycation/FGF21-covered substrates. It was proven that FGF21 is not toxic to any of the examined cell lines. The obtained results can serve as useful reference systems for designing complexes that can extend the protein's half-life in its active state.

## Novel composites of smectite minerals and TiO<sub>2</sub> 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)

Titania nanoparticles used for the preparation of TiO<sub>2</sub>/smectite composites were obtained by means of the inverse microemulsion method. An inverse microemulsion is a thermodynamically stable and optically transparent dispersion of aqueous microdroplets in a continuous oil phase, stabilized by the presence of surfactant molecules at the water-oil interface. The clay component, montmorillonite (Mt), was prepared in three different forms: as an organic derivative of Mt containing cetyltrimethylammonium cations in the interlayer region (CTA-Mt), as a sodium form of montmorillonite (Na-Mt) and as a hydrogen form of montmorillonite, obtained by activating Mt with hydrochloric acid (H-Mt). During the reporting period, the focus was on optimizing the photocatalytic properties of TiO<sub>2</sub> nanoparticles. It was found that they were highly dependent on the synthesis parameters. In particular, by modifying the composition of the aqueous phase of the microemulsion, it was possible to obtain TiO<sub>2</sub> nanoparticles that were either phase-pure anatase or rutile. The type of precursor (TiCl<sub>4</sub>, Ti alkoxide), and the medium used for washing the precipitate (water, water/ethanol, ethanol) were also very important for shaping the structure of TiO<sub>2</sub> nanoparticles. As a result of the research, a method was developed to synthesize TiO<sub>2</sub> nanoparticles with photocatalytic activity superior to the reference P25 photocatalyst (tested in the degradation of rhodamine B) (Fig. 1).

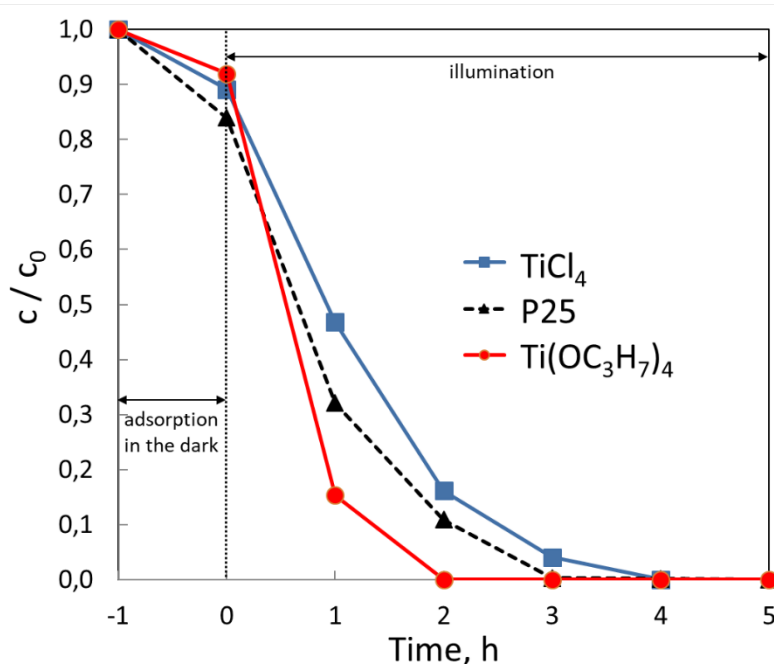


Fig. 1. Photocatalytic decomposition of rhodamine B ( $10^{-5}$  M) on TiO<sub>2</sub> nanoparticles obtained from different precursors (TiCl<sub>4</sub>, Ti(OC<sub>3</sub>H<sub>7</sub>)<sub>4</sub>) in comparison with the photoactivity of reference TiO<sub>2</sub> (P25).

## **Coarse-grained modeling of carbohydrates**

### **Parameterization of the MARTINI 3 force field for hyaluronan, investigation of the conformational properties of uronates, and comparative analysis of force fields for describing glycosidic bonds**

"Opus" research project NCN 2019/35/B/ST4/01149 [2020-2024]

(Project leader: Professor Wojciech Plaziński)

A model operating at the coarse-grained (CG) resolution level has been developed for simulating the molecular dynamics of hyaluronan and its building blocks (GlcNAc and GlcA). The developed model is compatible with the MARTINI 3 force field and a CG model previously derived within the same project, covering saccharides composed of glucopyranose units. The model was validated based on atomistic simulation results and structural data regarding hyaluronan-protein complexes. Additionally, a series of simulations were conducted to investigate interactions between hyaluronan and lipid membranes with varying compositions.

In studies related to the conformation of uronate monosaccharides, conducted at the quantum-mechanical level and through classical atomistic simulations, several results were obtained regarding the conformational equilibrium of the ring, the influence of metal ion presence on this equilibrium (particularly significant for sulfonated derivatives of iduronates), and the accuracy of the Karplus equation in the context of 3JHH coupling constants for all compounds in the iduronate group. The possibility of using hybrid potential simulations (QM/MM) to study conformational equilibria in monosaccharide molecules was also explored.

As part of an independent project component, calculations based on three different atomistic force fields (CHARMM, GLYCAM, and GROMOS) were initiated to compare their predictions primarily related to glycosidic bonding but also to understand how differences in the conformational properties of glycosidic bonds translate into the structural and conformational characteristics of longer chains. The studies encompass three types of glycosidic bonds:  $\beta(1-4)$ ,  $\alpha(1-4)$ , and  $\beta(1-3)$ . To date, a series of systems have been constructed, atomistic simulations have been conducted, and basic analyses have been performed.

## **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*)

In 2023, the tasks were continued on the development of the sample holder system allowing the MBE process to be carried out in UHV conditions, under the influence of external factors and for in situ magnetic and electric measurements. In particular, the holders for preparation in a magnetic field were modified by improving the magnet cooling system, which allowed the preparation temperature to be increased. Test measurements of electrical resistance as a function of thickness were performed during the growth of epitaxial Fe films on MgO(001).

The significant impact of the magnetic field applied during growth on the magnetic properties was shown for ultra-thin epitaxial magnetite films grown on MgO(111). Using in situ and ex situ characterization methods, scanning tunneling microscopy, conversion electron Mössbauer spectroscopy, and the magneto-optic Kerr effect (MOKE), we showed that the in-plane magnetic field applied during the reactive deposition of 10-nm Fe<sub>3</sub>O<sub>4</sub>(111)/MgO(111) heterostructures influenced the growth morphology of the magnetite films, which affected both in-plane and out-of-plane characteristics of the magnetization process. The observed changes were explained in terms of modification of the effective magnetic anisotropy.

The influence of the magnetic field on the growth and magnetic properties of epitaxial Fe(001) layers deposited on MgO(001) was also investigated. During the growth and annealing of the Fe films a magnetic field of 100 mT was applied in the film plane. As in the case of magnetite layers on MgO(111), in situ scanning tunneling microscopy measurements showed that the magnetic field had a strong influence on the film morphology, in particular on the structure of surface steps and terraces. The magnetic properties of the films were studied ex situ using magnetometry and MOKE microscopy. It was shown that the moderate in-plane magnetic field applied during growth has the visible impact on the magnetic properties. The observed angular dependence of the MOKE loops and domain structures were discussed based on magnetization reversal models. It was found that magnetization reversal occurs via 90° domains and the reversal differs for the no-field and in-field grown samples, in correlation with the film morphology.

## **New generation theranostatic 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 2023, we focused on the modification of polymeric or nanoemulsion core polyelectrolyte shell nanocarriers (core@shell nanocarriers) of selected neuroprotective drugs, cyclosporine A (CsA) and tacrolimus (FK506) for theranostic application. Drug-loaded nanocores (both types) were modified by sequential deposition of polyelectrolytes with gadolinium-labeled poly-L-lysine (PLL-Gd) for MRI as well as with rhodamine-labeled poly-L-lysine (PLL-Rod) for fluorescent imaging. The size of prepared nanocarriers ranged between 80-150 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.

## 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: Associate professor Jan Zawala)

The project is aimed to elaborate the fundamentals of simple physicochemical method of detection of surface-active contaminants (SAC) of waters, based on monitoring of changes of dynamic properties of liquid/gas interfaces. In practice, for SAC concentration determination in aqueous systems, the analysis of parameters of a single gas bubbles motion will be used, due to the fact that liquid/gas interface properties under dynamic conditions are extremely sensitive for even traces of organic substances in solution (of order of ppm and below).

During the reporting period, work was initiated to design and construct a prototype system intended for automatic measurement of the speed of a floating bubble in a liquid (aqueous solution). A prototype of such a device was made, which included a glass capillary with a defined inner diameter, an automatic generator of single bubbles, a glass column with a square cross-section and a housing with built-in phototransistors, which was specially designed for this purpose and made using 3D printing. The first prototype allowed for the measurement of the terminal velocity of a bubble floating in clean water with satisfactory accuracy, thus confirming that this methodology should be further developed.

Moreover, using a modernized set for measuring the bubble movement using the optical method (a high-speed camera, an automatic lifter and a script analyzing the collected images), measurements were made to determine the impact of the presence of organic phase vapors on the rate of establishment of a dynamic adsorption layer (DAL) on the surface of the floating bubble. in surfactant solutions. For this purpose, a bubble was generated using a syringe half-filled with hexane. This caused the bubble formed in the solution to be filled with hexane vapors, which changed the adsorption balance, leading to a much faster establishment of DAL on the bubble surface, compared to the situation in which the bubble was filled only with air. Moreover, in pure water, there was no effect of hexane vapors on the hydrodynamic boundary conditions on the bubble surface, which remained completely mobile, regardless of the presence of organic phase vapors. Example results of the velocity profiles of a bubble floating in pure water and in a n-octanol solution with a concentration  $1 \times 10^{-5} \text{ mol/dm}^3$  in the presence and absence of hexane vapors, shown in Fig. 1.

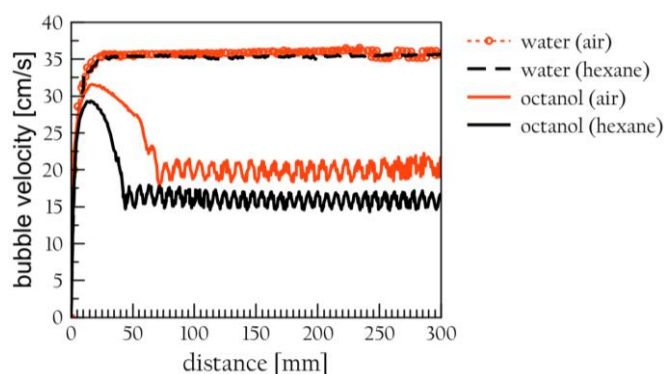


Fig. 1. Profiles of velocities of a single bubble filled with air or mixed air and hexane vapour, rising in pure water and n-octanol solution of concentration  $1 \times 10^{-5} \text{ mol/dm}^3$ .

## 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, IkiFP PAN partner: Associate professor Dorota Rutkowska-Żbik)

In this project we 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.

In the past year, the aim of the theoretical research was to compare descriptors obtained for the same state of the N-electron molecule, calculated using different methods. The following descriptors were selected: charge density, Laplacian density, electron localization function (ELF), localization and delocalization indices. The geometries of the formaldehyde molecule, selected as the test molecule, were optimized in the framework of density functional theory (DFT) using four functionals: CAM-B3LYP, M06-2x, TPSSh and  $\omega$ B97x as well as with the coupled cluster theory with single and double excitations (CCSD). For the determination of excited singlet and triplet states, calculations were performed using the Equation of Motion Coupled Cluster with Singles and Doubles (EOM-CCSD) method and the Time-Dependent Density Functional Theory (TD-DFT) approach. The aug-cc-pVDZ basis set was employed for the optimization process and excited states calculations. All geometries and energies were computed using the Gaussian16 program. The generation of wfx files, required for QTAIM (Quantum Theory of Atoms in Molecules) analysis of the electron density, was carried out using the Gaussian program. Subsequently, the AIMALL program was utilized for the actual QTAIM analysis.

The following electronic states of formaldehyde were characterized: the ground singlet state  $S_0$ , the first excited singlet state  $S_1$  and the first excited triplet state  $T_1$ . The calculated excited states can be described as resulting from the excitation of an electron located on the non-bonding electron pair of the carbonyl group onto the anti-bonding  $\pi^*$  orbital of the C=O bond. This process weakens and lengthens the C=O bond in the formaldehyde molecule in the excited state (both in  $S_1$  and  $T_1$ ). Moreover, it causes geometric changes within the carbon atom, which can be described as a change in geometry from planar to tetrahedral and an accompanying change in the hybridization of the C atom from  $sp^2$  to  $sp^3$ .

## 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, co-investigator, Agnieszka Kamińska PhD., Magdalena Szota PhD)

Due to their structure and high degree of monodispersity, polyamidoamine dendrimers (PAMAM) may constitute a promising nanocarrier for targeted drug delivery (DDS). The branched structure of dendrimers allows the formation of stable complexes with ligands through encapsulation in internal spaces or immobilization on the surface of the polymer structure. The location of the drug molecules has a significant impact on the kinetics of the release of the active substance and the mechanism of internalization of the carrier into the cell. As part of the conducted research, the conditions for the formation of effective complexes of the G4.0 PAMAM dendrimer with tautomeric form selected anticancer drugs were optimized: 5-fluorouracil (5FU) and doxorubicin (DOX). The study monitored the influence of the degree of ionization of the G4.0 PAMAM dendrimer molecule and anticancer drugs on the efficiency of complex formation using UV-Vis and NMR spectroscopy. The presence of drugs in the carrier causes changes in the zeta potential of the complex concerning the initial system. These changes are the result of electrostatic interactions between drug molecules and external functional groups. Using the DLS method, the stability of the complexes, their size, and possible degree of aggregation were monitored. The most favorable conditions for the formation of the G4.0 PAMAM-5FU and G4.0 PAMAM-DOX complex are a low degree of protonation of the dendrimer molecule with the simultaneous presence of the drug in a deprotonated form (LCG4.0-5FU=18.0%, LCG4.0-DOX=39.2%).

The activity of the complexes in vitro was determined using MTT tests. Tests showed the lack of toxicity of PAMAM dendrimers and the cytotoxicity of the complexes towards melanoma (A375), colon cancer (HT29), glioma (SNB-19), prostate cancer (Du-145) and breast cancer (MCF) cancer cells. Moreover, increased selectivity of the action of the complexes compared to drugs unrelated to the dendrimer was observed.

M. Szota, P. Wolski, C. Carucci, F.C. Marincola, J. Gurgul, T. Panczyk, A. Salis, B. Jachimska, Effect of Ionization Degree of Poly(amidoamine) Dendrimer and 5-Fluorouracil on the Efficiency of Complex Formation- A Theoretical and Experimental Approach. *Int. J. Mol. Sci.* 2023, 24, 81

M. Szota and B. Jachimska Effect of Alkaline Conditions on Forming an Effective G4.0 PAMAM Complex with Doxorubicin, *Pharmaceutics* 2023, 15(3), 875.



## New generation of multi-charge surfactants of dedicated functionality

"Opus" research project NCN OPUS 2022/45/B/ST4/01184 [2023-2027]

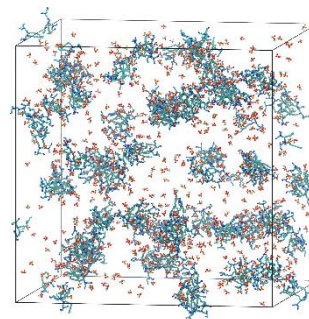
(IKiFP PAN partner: Piotr Warszyński, project coordinated by prof. K.A. Wilk, Wrocław University of Science and Technology)

The main goal of the project, carried out by a consortium of two research groups (from the Jerzy Haber Institute of Catalysis and Surface Chemistry PAS and the Faculty of Chemistry of the Wrocław University of Science and Technology), is to develop the scientific basis for an innovative strategy for the design and production of new multi-charge surfactants with dedicated functionality and to evaluate their usefulness in selected applications. In the project, surfactants with various hydrophilic groups with a dendritic structure are synthesized. Molecular dynamics methods combined with an approach based on thermodynamic models of adsorption of multi-charge surfactants make it possible to explain the mechanism of some phenomena observed experimentally and constitute the basis for the development of new materials intended for specific applications. The project combines theoretical description and experimental verification of the influence of parameters such as the number and type of hydrophilic groups and the length of the hydrocarbon chain on the adsorption and aggregation properties of newly synthesized surfactants.

In 2023, we measured the surface tension isotherms of a number of newly synthesized surfactants, the structures of which are presented in the table.

Surfaktantu	Structure
2-dodecyl- <i>N,N,N,N',N',N'</i> -hexamethylpropan-1,3-ammonium dibromide	
2,2'-(dodecylimino)bis( <i>N,N,N</i> -trimethyletanammonium) dibromide	
<i>N,N,N</i> -bis(2-( <i>N,N,N</i> -bis(2-ammoniummethyl)amino)ethyl)dodecylamine tetrabromide	
<i>N,N</i> -ethane-1,2-diylbis( <i>N</i> -dodecylpropane-1,3-bis( <i>N</i> -(3-aminopropyl)propane-1,3-diamine)-diamine) tetramethylsulfonate	

Aggregation simulations were also performed using the GROMACS ver. 2022.3. with the CHARMM36 force field and the OPC4 water model. An example snapshot obtained during the simulation is shown in the drawing. Aggregation structures of the surfactant *N,N*-ethane-1,2-diylbis(*N*-dodecylpropane-1,3-bis(*N*-(3-aminopropyl)propane-1,3-diamno)-diamine) surfactant are visible.



## 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)

Experimental work focused on three homologues of L-DOPA extradiol dioxygenases (L-DOPA 4,5-dioxygenase, DODA): *Escherichia coli*, *Portulaca grandiflora*, *Beta vulgaris*. The aim of the research was to obtain, using X-ray crystallography, the structures of DODA enzymes in the form of an apoenzyme (if such a structure has not yet been obtained, as in the case of *P. grandiflora*) or in a complex with selected ligands (*E. coli*, *B. vulgaris*). The following catechols and catechol analogues were tested: L-DOPA, epicatechin, epigallocatechin, quercetin, dopamine, norepinephrine, 4-nitrocatechol, mimosine. We managed to obtain data corresponding to the complexes BvDODA-Ni<sup>2+</sup>-4-nitrocatechol, BvDODA-Ni<sup>2+</sup>-mimosine, tEcDODA-Ni<sup>2+</sup>-4-nitrocatechol (in the case of the latter, better data quality was obtained than before). In order to increase the efficiency of DODA enzyme production from *P. grandiflora* and *B. vulgaris*, a new purification strategy was tested (one of the changes in the protocol was the use of homogenization), which actually allowed to obtain more milligrams of protein / 1 L of medium.

In the case of the enzyme ectoine synthase from *Sphingopyxis alaskensis*, samples were prepared for measurements using the Mossbauer method (SaEctC with <sup>57</sup>Fe), the aim of which was to better understand the arrangement of the active site and validate the computational results. Additionally, attempts were continued to obtain the structure of SaEctC with a bound metal ion (Fe or analogues, such as Co) and a substrate.

The computational work was aimed at understanding the probable mechanism of the reaction catalyzed by EctC. The results of QM/MM calculations and Mossbauer measurements suggest that the Fe<sup>2+</sup> ion bound in the active site is coordinated by 6 ligands and exists in the high-spin state (quintet). The determined free energy profiles along the reaction pathway revealed that the rate-limiting step of the process is the nucleophilic attack of the amino group on the carbonyl carbon of the substrate. The iron ion performs several catalytic functions: it activates the carbonyl group of the substrate, binds the OH group, which is a proton acceptor at the stage of C-N bond formation, and binds the tyrosine group, which supplies a proton to the oxygen atom of the substrate. The latter is then eliminated from the ectoine precursor as an OH group, which is bound by the iron ion.

## 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)

In the project, I want to solve the fundamental research problem regarding the impact of adsorption processes on dispersion system phase parameters and develop new and safe foams/emulsion substitutes with excellent biocompatibility and biodegradability alongside their manufacturing technologies to reduce the negative impact of traditional chemical surfactants.

To achieve these goals, I must clarify the impact of molecular structure and effects of dynamic adsorption of surfactants at different hierarchical levels of aqueous functionalised foam and emulsion from the nano to the macroscopic scale. Therefore, in the project's first year, I conducted a series of tests using a safe model surface-active compound (saponin), which interacted with compounds that were hydrogen bond donors or acceptors.

It was found that, for example, the addition of urea, which can be both a donor and an acceptor of hydrogen bonds, reduces the surface activities of saponin solutions. On the contrary, adding choline, the only acceptor of hydrogen bonds, enhanced the surface activity properties of the tested saponin solutions. The same applies to glycerin, but the compound's influence on the solution's viscosity should also be considered in this case.

In the work, a series of measurements were made for mixtures of all the tested compounds, saponin, urea, choline and glycerin, to examine the chemical mutual interactions. Thin-film stability (Thin Liquid Film Pressure Balanced Technique), foam structure and rheology (visco-elastic) analysis of foam fraction tests are planned for the following year.

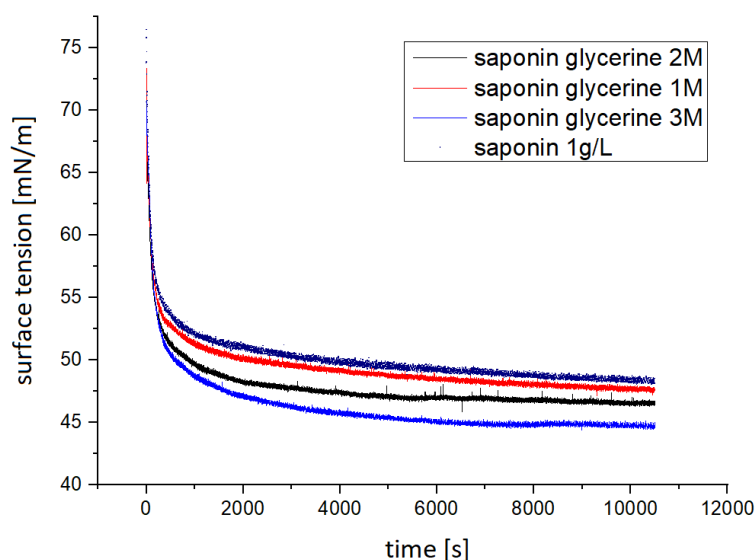


Fig. 1. Changes in surface tension in saponin solutions and its mixtures with glycerol.

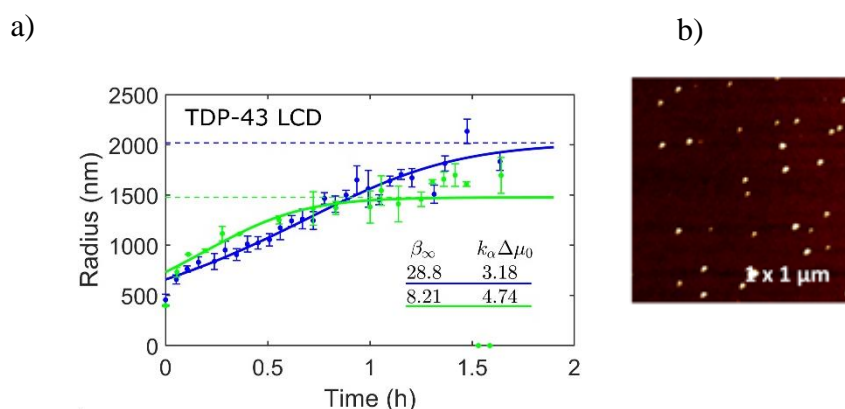
# From Single Molecules to Stress Granules – Understanding the Mechanisms of Phase Separation of Proteins linked to Amyotrophic Lateral Sclerosis

"Opus" research project NCN 2021/43/B/ST8/01900 [2023-2027]

(Project leader: Anna Bratek-Skicki DSc, contractors: Z. Adamczyk, P. Żeliszewska, A. Kluza)

Neurodegenerative diseases affect nearly 50 million people around the world, and this number is constantly growing as people live longer and the number of risk factors increases. They are characterized by progressive degenerative changes in nerve cells, which become more damaged and dysfunctional over time. One example of such disease is amyotrophic lateral sclerosis (ALS), a fatal disease of motor neurons. Patients' nerve cells contain protein aggregates whose presence is associated with their toxic effect on neurons. They are often formed during a process called phase separation, which, under pathological conditions, leads to the formation of aggregates. Currently, there are no effective drugs or therapies to treat ALS.

The main goal of the project is to understand the mechanisms responsible for the formation of stress granules (condensates) (SG) and their pathological transformation towards the formation of aggregates made of ALS-related proteins. This project is mainly focused on proteins such as TDP-43, hnRNPA2, which are present in ALS patients, G3BP1, a protein responsible for initiating the formation of SGs, and FUS, a multifunctional protein also involved in many metabolic pathways. Understanding these processes will allow us to control the formation of condensates, which in the long run may contribute to the development of effective therapies against this disease. In 2023, the focus was on the physicochemical characterization of TDP-43 and hnRNPA2 in solution under various pH and ionic strength conditions and the study of kinetics of phase separation (Fig. 1a). AFM measurements were also performed to determine the size distribution of protein molecules, followed by their adsorption on negative latex to determine the charge and isoelectric point. It was shown that TDP-43 and hnRNPA2 form stable oligomeric structures in MES and CAPS buffers before the initiation of phase separation (Fig. 1b). Moreover, in cooperation with the group from the University of Porto in Portugal and the group from the University of Brussels (VUB) in Belgium, a model of phase separation was developed, taking into account the role of surface tension during the formation of the first condensate nuclei and their further growth. It has been shown that the formation of a limited number of large condensates is possible without active mechanisms of size control and in the absence of coalescence phenomena. The exact analytical solution can be used to interrogate how the elementary steps of phase separation are affected by candidate drugs.



**Fig.1.** Physicochemical characterization of TDP-43 (phase separation kinetics, adapted from Z. Sárkány et al., Adv. Sci. 2023, 10, 2301501) and hnRNPA2 (protein in CAPS buffer): a) growth of condensates formed from TDP-43 as a function of time measured by dynamic light scattering, b) oligomeric structures of hnRNPA2 imaged by AFM.

## 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 (metal – metal distance equals to ca. 7.5 Å) bi-nuclear site, for the O-O bond activation in O<sub>2</sub> and formation of the reactive oxygen species responsible for selective oxidation in model systems; 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 O<sub>2</sub> activation by two distant cooperating Fe(II) ions was studied using the model consisting of two iron(II) porphyrin species which were placed in parallel one to another at a distance of 7.5 Å. The dioxygen molecule was placed in between the two iron(II) 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 O-O distance was selected as the reaction coordinate and the potential energy scan was then performed, followed by the transition state optimization. The calculations were done within Density Functional Theory using Turbomole v6.3. def2-TZVP basis set was used with Perdew-Burke-Ernzerhof (PBE) functional. Resolution of Identity (RI) approach, known also as density fitting, was applied for computing the electronic Coulomb interactions. In the transition state, the O-O bond in the dioxygen molecule is elongated from 1.21 Å (the bond length in the isolated O<sub>2</sub> molecule) to 1.57 Å. Both iron ions bind oxygen atoms (Fe-O bond lengths equal to 1.94 Å and 1.95 Å). The process is accompanied by a significant iron ions out-of-plane displacement accompanied by the deformation of the porphyrin rings. Then, two high-valent iron-oxo porphyrins are produced along the reaction. The barrier for the dioxygen splitting equals to 38 kcal/mol. The Fe=O bond lengths equal to 1.625 Å, the same distance between iron and oxygen in both groups is found. The distance between the oxygen atoms amounts to 3.80 Å. The current calculations demonstrate the ability of distant divalent binuclear iron sites to activate and split dioxygen by its splitting despite the nature (aluminosilicate, organic) of the “matrix” employed for the stabilization of Fe(II) ions under proper distance and geometrical arrangement of the Fe(II) ions.
- 2) The catalytic reaction (oxidative dehydrogenation of propane) was run in a fixed-bed gas flow reactor with a gas chromatograph to detect substrates and reaction products at a temperature range from 200-500 °C, with varying contact times. The reaction conditions were optimized, and the preliminary testing of the studied systems were done.

## 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, Investigators: Sonia Bujok PhD. Eng., Sergii Antropov PhD)

This project aims to develop a preventive conservation strategy to determine the most convenient economic and ecological conditions for storing cultural heritage objects made of poly(vinyl chloride) – PVC. Research within this project is mainly focused on following the process of degradation under mild conditions (maximum temperature 80 °C) via observation of chemical changes in PVC and their correlation with the mechanical changes of the material.

In the second year of the project, research on the migration of plasticisers was finalised and the diffusion and surface emission coefficients of the most common plasticiser in heritage objects, i.e. DEHP (bis(2-ethylhexyl) phthalate), were determined. Moreover, a function describing the DEHP loss from objects over time depending on the initial plasticizer content, temperature and thickness of the object was proposed. Based on this, the most vulnerable objects in PVC collections were defined as thin films or objects covered with a layer of plasticised PVC. It was found that the airflow speed (ventilation) did not significantly affect the rate of plasticizer loss. It was also shown that removing the plasticizer layer from the surface of the object does not change the speed of its migration, which allowed us to conclude that cleaning objects made of plasticized PVC is a safe conservation procedure. The model of plasticiser migration developed using experimental data and computer simulations was refined using the results of a survey of the PVC collection. Moreover, diffusion simulations of the smallest (most mobile) phthalate plasticiser, i.e. DBP (dibutyl phthalate), were performed using molecular dynamics in collaboration with Prof. Tomasz Pańczyk. Additionally, the DBP diffusion coefficient was determined using NMR diffusometry in a solid state in cooperation with the NanoBioMedical Center (UAM, Poznań).

Together with a team from the Faculty of Chemistry of the Jagiellonian University, a non-destructive method for the qualitative analysis of plasticisers in cultural heritage objects was proposed based on the analysis of Raman spectra using machine learning. Accelerated ageing experiments of rigid (unplasticised) and plasticised PVC at 60 and 80 °C were completed, and a series of analyses were carried out to correlate chemical changes (spectroscopic techniques: Raman, infrared, UV-Vis, and electron paramagnetic resonance; X-ray fluorescence, X-ray photoelectron spectrometry, scanning electron microscopy with energy dispersive spectroscopy, contact angle measurement, size exclusion chromatography) with changes in mechanical properties (static tensile test, dynamic mechanical analysis).

The mechanical model for an inflatable object made of plasticised PVC was developed in the COMSOL Multiphysics® software based on the material parameters determined for various types of PVC in static and dynamic tests, which enable the prediction of the long-term mechanical response of the material. The model will be used to assess the risk of mechanical damage (deformations and cracks) in PVC heritage objects.

Based on the above experiments and computer simulations, guidelines for preventive conservation for contemporary art objects made of or containing elements of plasticised PVC will be proposed.

**"Sonatina" Research Projects  
of the National Science Centre**





## Multifunctional chitosan-based composite coatings for biodegradable Mg alloys

"Sonatina" research project NCN 2021/40/C/ST5/00266 [2021-2024]  
(*Project leader: Dzmityr Kharytonau PhD*)

Magnesium alloys are promising for use in biodegradable materials. Despite major 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 second and the third specific objectives of the project have been examined:

Task 2. Fabrication and characterization of chitosan-based composite coatings on the surface of Mg alloys.

Three types of chitosan powders with high, medium, and low molecular weight were selected for experiments. As second-phase particles were selected ZnO, hydroxyapatite, and halloysite nanotubes. For coordination deposition phosphate and molybdate ions were selected. Obtained coatings were characterized by X-ray diffraction (XRD) analysis, scanning electron microscopy (SEM), FT-infrared spectroscopy, and Raman spectroscopy. As substrates, Mg alloys AZ31 (Mg-Al-Mn) and WE43 (Mg-Y-Nd-Zr) were selected. Based on the obtained results, promising coatings were selected for further tests.

Task 3. Analysis of mechanical properties of coatings.

In terms of Task 3, an international internship under the supervision of Prof. Nils Almqvist has been started 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 are 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 are currently being investigated.

Additionally, an in-depth examination of the corrosion properties of AZ31 and WE43 alloys in Hank's solution was performed. A multisine impedance-based tool for monitoring non-stationary electrochemical processes, coupled with distribution of relaxation times (DRT) analysis was proposed to examine the corrosion mechanisms of these alloys.

To compare the performance of chitosan coatings with other biodegradable coatings, a series of polylactic acid (PLA) and phosphate coatings were prepared on the surface of the WE43 Mg alloy. Their microstructure and phase composition were examined and protective performance was evaluated in Hank's solution.



**"Preludium Bis" Research Projects  
of the National Science Centre**



## **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*)

Neurodegenerative diseases are incurable and therefore the attention of scientists is focused on the determination of their pathogenesis and etiology, and on the development of methods for their treatment and prevention. Many literature reports provide evidence that unwanted protein aggregation into fibers (fibrils), resulting from faulty protein folding, is associated with the etiology of neurodegenerative diseases. It has been demonstrated that certain metal nanoparticles and metal oxides, which are nanometric structures with sizes smaller than 100 nm, exhibit activity in protein fibrillation processes. The activity of nanoparticles in protein fibrillation processes depends on their physicochemical properties, primarily the chemical structure of the stabilizing layer.

The main aim of the proposed project is the development of methods of functionalization of selected metal (Ag, Pt) and metal oxide (TiO<sub>2</sub>, Fe<sub>3</sub>O<sub>4</sub>, ZnO) nanoparticles by low molar mass polyphenols of well-documented activity in the fibrillation processes and the evaluation of their impact on the fibrillation processes of proteins and peptides Gallic acid and rosmarinic acid belong to the low-molar mass of polyphenols of well-documented activity in the fibrillation processes. Moreover, both selected polyphenols are antioxidants of antibacterial and antiviral properties. Some literatures reports have shown that these polyphenols also are able to inhibit cancer development. The main hypothesis assumes that the functionalization of metal and metal nanoparticle surfaces by these polyphenols of anti-fibrillar properties will inhibit fibrillation processes more strongly than free polyphenols and nanoparticles.

In the first stage of the research, the attention was focused on obtaining silver nanoparticles (AgNPs) stabilized with gallic acid and rosmarinic acid. A series of syntheses was carried out, in which AgNPs were obtained by the reduction of silver ions using sodium borohydride in the presence of either gallic acid or rosmarinic acid. In the second approach, both polyphenols served as both reducing agent and stabilizers for AgNPs. The obtained AgNPs hydrosols underwent a purification process to remove excess unreacted reagents, followed by the characterization of their physicochemical properties.

Based on microscopic imaging (SEM, TEM), it was determined that, regardless of the applied procedure, AgNPs exhibited a quasi-spherical shape with sizes ranging from 5 to 30 nm. Synthesis condition optimization revealed that increasing the concentration of polyphenols and/or the pH of the reaction mixture resulted in smaller-sized nanoparticles.

Spectrophotometric measurements indicated that, regardless of the preparation procedure, the stabilization of AgNPs resulted from the adsorption of the applied polyphenols at the solid/liquid interfacial boundary. Measurements using dynamic and electrophoretic light scattering techniques demonstrated that all types of obtained AgNPs underwent aggregation under acidic conditions (pH < 3.5) and ionic strength higher than 10 mM. Both AgNPs stabilized with gallic acid and rosemary exhibited a negative zeta potential, the value of which decreased with increasing pH. None of the obtained nanoparticles exhibited an isoelectric point.



**"Preludium" Research Projects  
of the National Science Centre**





## Tungsten-containing aldehyde oxidoreductase from *Aromatoleum aromaticum* - study of catalytic reaction mechanism

“Preludium” research project NCN 2017/27/N/ST4/02676 [2018-2023]

(Project leader: Agnieszka Winiarska MSc; supervisor: Professor Maciej Szaleniec)

Tungsten aldehyde oxidoreductase (AOR) enzymes catalyze a reversible oxidation of aldehydes to the corresponding carboxylic acids in the presence of ferredoxin, and because of their broad substrate spectrum, they are assigned a role in detoxifying harmful aldehyde metabolites in the fermentative degradation of amino acids. The tungsten aldehyde oxidoreductase from *Aromatoleum aromaticum* (AOR<sub>Aa</sub>) was chosen for the study because of its high oxygen resistance and lower temperature optimum than known AORs from hyperthermophilic archaea (i.e. *Pyrococcus furiosus*) and bacteria.

The research planned in the project was aimed at providing knowledge about the structure of the enzyme's active center and its reactivity, which will make it possible to determine the mechanism of the reaction catalyzed by AOR<sub>Aa</sub>. Structural characterization of AOR<sub>Aa</sub> was performed by cryo-electron microscopy and mass photometry, which showed that the protein forms a filament of AorAB protomers capped by a single AorC subunit (Fig. 1). The AorA subunit oligomerizes into an electron-conducting nanowire connecting the enzymatically active AorB subunits containing a tungsten cofactor (W-co). Structural details provided by the reconstructed 3.3 Å resolution AOR<sub>Aa</sub> density map showed the binding mode of benzoic acid at the active site.

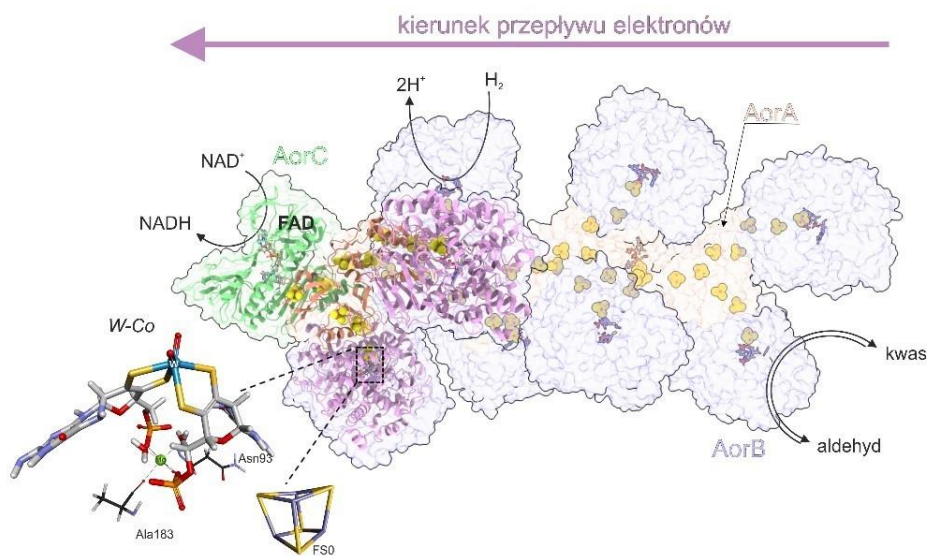


Fig. 1. Reconstruction of the oligomeric structure of AOR<sub>Aa</sub> based on the structure from CryoEM, mass photometry measurements and QM:MM calculations.

To prove that the C-terminal fragment of AorA plays the main role in filament formation, a variant of the  $\Delta$ helix-AOR protein was constructed without AorA residues from 138 to the C-terminus. Then, only AorABC complexes were observed using mass photometry, confirming the hypothesis about the key role of the C-terminal helix in the enzyme's filamentation.

## **The analysis of the influence of selected factors on changes in $\alpha$ -synuclein structure**

“Preludium” research project NCN 2020/37/N/ST4/02132 [2021-2024]

*(Project leader: Paulina Komorek MSc, supervisor: Professor Barbara Jachimska)*

Parkinson's disease is a neurodegenerative disease that affects approximately 2% of the population over 60 years of age. It is mainly characterized by disorders of the musculoskeletal system and is mainly manifested by slowness of movement, tremors, muscle stiffness, and loss of coordination. A characteristic feature of the disease is the presence of Lewy bodies - abnormal protein aggregation that develops inside nerve cells. Studies of the composition of Lewy bodies have shown that they mainly contain the protein  $\alpha$ -synuclein, which occurs in incorrectly folded forms and may cause neuronal death. In this case, a lot of effort has been put into research on  $\alpha$ -synuclein. It has been shown that disruption of long-range interactions between  $\alpha$ -synuclein regions may cause incorrect folding of the polypeptide chain. One of the factors that can induce these changes is phenomena occurring at the phase boundary. Thermodynamic and kinetic studies at the phase boundary confirmed that conformational changes may occur when a protein molecule comes into contact with the surface of a biological membrane. Another factor that may disrupt the interaction between  $\alpha$ -synuclein regions is a change in the charge distribution on its surface. Experimental studies confirm that even a slight change in the charge distribution on the protein surface is an indicator of changes in the secondary structure of  $\alpha$ -synuclein, even leading to aggregation. It should be emphasized that current research mainly concerns the behavior of  $\alpha$ -synuclein in solution, much less research is devoted to the interaction of this protein with the surface, which may be of key importance in the process of changes occurring in the secondary structure.

Research conducted as part of the project concerns the impact of molecular interactions of disordered proteins with the surface on the secondary structure of the protein and the efficiency of protein deposition on a selected model surface with a known surface charge density. The research contributes to the elucidation of the mechanism of  $\alpha$ -synuclein folding towards the formation of toxic aggregates and fibrils.

## **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 Bratacz)

As part of the project, work was continued on 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. In particular, the project aims to define a safe strategy for heating historic interiors for religious, cultural, and commercial short-term events.

The results obtained from measurements of the mechanical properties of lime wood were analyzed: Young's moduli in the tangential and radial directions, Poisson's ratios, critical energy release rate and strain at break. These values were expressed in relation to the water content in wood (MC) and then converted to relative humidity (RH) using the scanning isotherm equation. For the purposes of modeling, functions describing the variability of parameters in the full RH range (0-100%) were fitted to the obtained data due to the significant annual RH amplitude in historic interiors.

A two-dimensional model of a massive wooden element, allowing to analyze the crack propagation process due to climate fluctuations, was adapted to estimate risks in historical buildings in Central Europe by updating the mechanical properties in the model with experimentally obtained values. Then, the previously conducted preliminary numerical simulations of changes in the energy release rate (G) because of a sudden drop in RH and as a result of cyclic changes were assessed in the light of the new data obtained on the properties of wood. As a result of cyclic changes described by the sine function, an increase in the RH level corresponding to the lack of stress in the material was observed in relation to the original RH level around which the sine function oscillated.

A full simulation of the climate of a historical building interior - St. Mary's Basilica in Kraków - was also carried out and it was based on the T/RH measurements conducted in the church. To assess the threat that this variability may pose to the Veit Stoss' Altar, a lime wood sculpture with a diameter of 1 m was modeled.

An experiment was planned to validate the modeling results, in which discs of lime wood are subjected to a step change in RH in a climatic chamber. Verification is based on visual inspection of crack growth resulting from drying combined with very sensitive detection of damage development using the acoustic emission method. In the first step, the RH level at which there are no stresses in the disks was determined, which is the starting point of the experiment. For samples obtained from wood seasoned for 2 years, this level corresponded to approximately 90% RH. Samples stabilized in these conditions were subjected to a decrease in RH to 50% in the next step.

In order to determine the correlation between the number of energy units recorded by the acoustic emission method and the crack growth, the results obtained during wedge-splitting measurements were analyzed in this respect. Linear dependence curves of these two parameters were found, which will be used to estimate the development of damage invisible to the eye in the monitoring of real objects.

## **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 Zawala*)

As part of the project, the synthesis of magneto-reactive nanomaterials with a modified surface, core-shell type, where magnetite was used as the core and silica as the outer layer, was carried out. The obtained materials were thoroughly characterized in terms of size and morphology (SEM, AFM, DLS) and chemical composition (UV-VIS, FT-IR, RAMAN, XPS). The synthesized materials were used in synergistic mixtures with the amphoteric surfactant C12-Arg, for which adsorption properties were determined by surface tension measurements and SFG spectroscopy. Tests for pure surfactant have shown that the surface properties (surface tension measurements) of the surfactant vary depending on pH. This fact was justified by the amphoteric properties (cationic and anionic forms) of the surfactant, confirmed by SFG spectroscopy measurements. The properties of synergistic mixtures based on the amphoteric surfactant C12-ARG and selected types of nanomaterials were carried out using surface tension measurements and SFG spectroscopy. In the case of SFG measurements, they were additionally performed using an external magnetic field. Studies have shown that in the case of systems where the surfactant is oppositely charged to the nanoparticles at a given pH, a synergistic reduction in surface tension and changes in signals in the SFG spectra are observed compared to pure mixtures. SFG measurements using a magnetic field showed the dependence of this effect on the applied external magnetic field. The stabilizing properties of the foam film as well as the influence of the magnetic field were determined using dynamic interferometry of the foam film. The experimental work carried out demonstrated the adsorption synergistic properties of the tested systems depending on the external magnetic field. In further research, the foaming properties of pure surfactant and mixtures with nanomaterials at selected pH were also checked using the dynamic foam analysis method. Research has shown that the addition of particles with the opposite charge to the surfactant at a given pH leads to increased foamability and stability of the foam created under given conditions.

**"Beethoven Life" Research Projects  
of the National Science Centre**



## 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*)-benzylsuccinate.

The conducted modelling allowed the finishing of the theoretical description of the reaction mechanism with QM:MM methods. As a result, a full reaction energy profile was obtained for the reaction with a monoprotonated fumarate. The calculations showed, that the barrier associated with the activation of cysteine is 55.9 kJ/mol, activation of toluene only 20 kJ/mol, with C-C bond formation 45.8 kJ/mol while the barrier associated with radical quenching is 60.5 kJ/mol. These results indicate, that the observed kinetic isotope effect, because of the relatively close energy of the barrier weakly sensitive to isotope labelling of toluene (i.e. C-C bond formation) and barrier highly sensitive to labelling (radical quenching), but which can be subjected to H/D exchange with the solvent.

Furthermore, calculations showed regioselectivity of attack on distal C atom in the C=C group of fumarate, for the fumarate bound in the proR conformation. This preference is reversed for the proS-bound fumarate. This factor explains the high enantioselectivity of the reaction which yields exclusively *R*-benzylsuccinate.

We also modelled the enantioselectivity of Cys493 activation by Gly829. The modelling was conducted for enzyme in complex with substrates and product as well as for apoenzyme. The calculations showed that for E:S and E:P activation process proceeds preferentially for proR conformation but the proS activation is also possible (only 22 kJ/mol difference between barriers). This result explains the observed experimentally H/D exchange of H atom at radical Gly for enzyme incubated in D<sub>2</sub>O which can be observed by EPR.

On the other hand for apoenzyme, the calculations showed that the activation of Cys proceeds slower in comparison to substrate or product bound complexes. Furthermore, the preferential H atom transfer proceeds in proS conformation. However, to enzyme's conformation has to change in order to enable proS transfer, and this is associated with the energy cost of 80 kJ/mol.

The experimental part of the project focused on the kinetic characterization of an inhibitory effect of benzaldehyde and benzyl alcohol on the reaction rate of reaction with toluene and fumarate. The kinetic tests were conducted with native BSS from *T. aromatica* K172. The kinetic tests were also conducted for native reaction with toluene yielding apparent kinetic constants. Initial experiments for xylenes were also conducted establishing methodology and analytical protocols. We also repeated the H/D exchange experiment with BSS incubated in D<sub>2</sub>O which led to a change in the EPR signal of glycyl radical. We have also optimized the purification of non-activated recombinant BSS produced in *E. coli* BL21 using magnetic beads functionalized with streptavidin. Such purified enzyme was characterized by mass photometry, which allowed optimization of experimental conditions for structural analysis of the enzyme with cryo-EM technique.





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



# 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 [2020-2023]  
(Project leader: Associate Professor Dorota Rutkowska-Żbik)

Using the DFT:PBE+D3/def2-SVP method in a cluster approach, the geometric and electronic structures of the catalysts tested in the photocatalytic methane coupling reaction were determined, i.e. M/HPA and M/HPA/TiO<sub>2</sub> systems (M = Ag, Au, Cu, Pd, Pt as electrically neutral atoms or ions in their typical oxidation states; HPA = HPW, HPMo), where a cluster cut from the (101) surface of anatase was used as a TiO<sub>2</sub> model. It was found that the HOMO orbitals of M/HPA systems consist mainly of transition metal orbitals, while the LUMO orbitals are located within heteropolyanions. In the next step, the reactivity of the above-mentioned systems towards the methyl group, benzene and carbon monoxide, which are reagents in methane conversion processes, was examined. All metals form thermodynamically stable bonds with the molecules studied, and metal ions have been observed to form stronger bonds than the neutral atoms.

Then, the geometry and electronic structure of the systems in which metallic copper in the form of single atoms and small clusters deposited on titanium dioxide was examined. As a result of deposition on TiO<sub>2</sub>, the metallic phase is oxidized - electrons from copper flow to the support, where they are located mainly on oxygen atoms in the immediate vicinity of Cu atoms. This charge reorganization causes new active centers to be created on the surface of the Cu/TiO<sub>2</sub> system: copper atoms acquire electrophilic properties, and oxygen atoms at the phase boundary become stronger nucleophilic centers compared to those present on the pure titanium dioxide surface.

Finally, the full mechanism of the methane to ethane coupling taking place on TiO<sub>2</sub> with an introduced oxygen vacancy was also investigated - the energy profile is shown in the Figure below. Calculations were performed using the DFT:B3LYP/LANL2DZ method. The limiting step is the breaking of the first C-H bond (the energy barrier of this process is 167 kJ/mol), the formation of the C-C bond involves a lower energy input (the barrier is 134 kJ/mol).

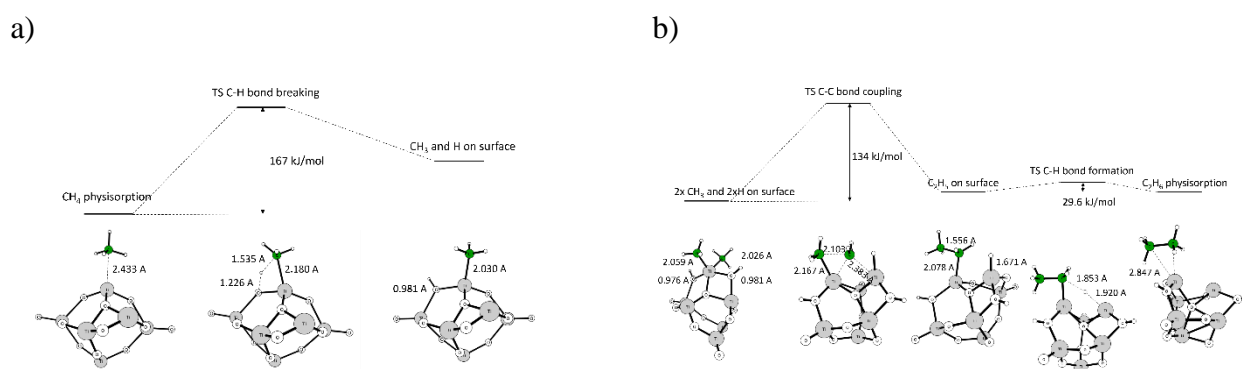


Figure 1. Energetic profile of CH<sub>4</sub> activation on TiO<sub>2</sub>: a) C-H bond activation in methane; b) coupling of methyl groups and ethane formation.



**"GRIEG" Research Projects  
of the National Science Centre**



## **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;*

*co-investigator: Marcin Bury PhD, Marcin Strojecki PhD, Arkadiusz Janas PhD, Sergii Antropov PhD, Katarzyna Poznańska MSc Eng.)*

In 2023, the research team implementing the GRIEG Craquelure project continued to develop a database of properties of historical and artistic materials used in paintings, and contemporary materials used to consolidate a paint layer. The measurements of elasticity modulus, strain at break, and swelling and shrinkage isotherms of tempera paints carried out in previous years were extended to determine the fracture toughness of tempera and oil paints.

The experimental work was made possible by the adaptation of the ASTM D5528 – 13 standard method to the size of available oil paints for a selection of oil paints from Mecklenburg's Paint Reference Collection now after approximately 30 years of drying. The new experimental procedure allowed to improve the statistics of the measurements but also to get insight into in-depth profiles of the fracture toughness of some paints. The fracture toughness  $G_{IC}$  values are the lowest for zinc white, raw umber and lead white with litharge, approximately 15 J/m<sup>2</sup> and the highest for sap green. The variable homogeneity of some paints – lead white, zinc white, sap green and Verdigris – is clearly reflected in the large range of the values obtained. Similar values were obtained for lead white, azurite and yellow ochre egg tempera paint. The results demonstrated that some paints are more brittle than the glue-based ground layer and as a consequence more vulnerable to cracking.

On the other hand, the 3D model of a painting was used to study the cracking of egg tempera and oil paints, laid on a gesso produced following historical procedures. Two scenarios of stress development were analysed: cumulative drying shrinkage of paints or gesso, owing to gradual loss of water or evolution of the molecular composition of the binders, and moisture-induced swelling of the wood substrate. Ratios of distances between cracks in the tangential and longitudinal directions of a wood panel to the layer thickness were estimated for increasing magnitudes of materials' dimensional response in the two scenarios. The critical values of the ratios for which stress in the midpoint between the cracks dropped below the value inducing strain at break in the materials and saturation of the crack patterns occurred, was approximately 3-4 or 5-6 for the paints and the gesso, respectively. The critical distance normalized to the gesso thickness between cracks parallel to the wood grain induced by swelling of the wood substrate due to relative humidity variation in the range of 50 – 70% was 6. The study demonstrated that crack spacings in the fully developed crack systems remain sensitive only to the thicknesses of paint or gesso layers which, therefore, can be derived from the crack pattern geometry. Existing flaws in gesso were found not to increase the risk of new crack development.

## 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

co-investigator: Associate professor Krzysztof Szczepanowicz, Marta Szczęch PhD, Tomasz Kruk PhD)

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 into 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 Norwegians SINTEF Oslo and Oslo University Hospital participate in the project consortium.

In 2023, we continue research on the encapsulation of carnosic acid (neuroprotective substance) into polyelectrolyte nanocarriers. The nanocarriers of carnosic acid were formed by the sequential adsorption of charged nanoobjects method, called layer by layer. Nanocarriers were modified for MR imaging as well as for optical imaging based on fluorescence. The external layer of nanocarriers was functionalized by the formation of PEG corona to allow long circulating after administration and reach the blood-brain barrier. The size of the developed theranostic nanocarriers was 150 nm. Figure 1 presents cryo-SEM images of developed nanocarriers. Developed nanocarriers of carnosic acid were delivered to project partners for in vitro and in vivo tests.

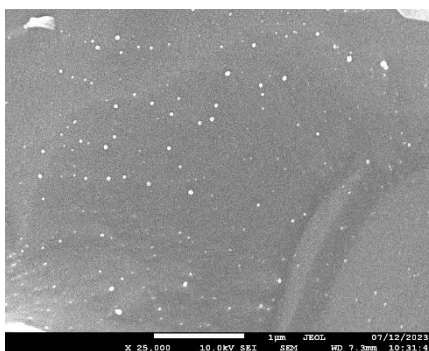


Figure 1. Cryo-SEM image of nanocarriers labelled with gadolinium.



**"Miniatura" Research Projects  
of the National Science Centre**



## **Effect of redox and acidobasic properties of the active sites on the activity of non-heme iron dependent enzymes - computational studies**

"Miniatura" research project NCN 2022/06/X/ST4/00078 [2022-2023]

(Project leader: Zuzanna Wojdyla PhD)

The project covers a short post-doctoral fellowship at the J. Heyrovský Institute of Physical Chemistry in the Computational Chemistry group, under the supervision of RNDr Martin Srnec. The aim of the fellowship was to develop a computational model of the pterin-dependent enzyme, which will be in line with the recent experimental findings, and will serve to describe the relationship between the redox and acidobasic properties of the active site of the enzyme and its activity.

The initial stage of the studies was based on classical modelling – docking of the cofactor (4a-hydroxytetrahydrobiopterin) and the substrate within the binding cavity of the enzyme, followed by molecular dynamics (MD) simulations for the obtained complexes. In the next step, the complexes most representative for the simulations were optimised with use of QM/MM methods and benchmarked with experimental results (by comparison of results of TD-DFT calculations with experimental UV-vis spectra).

To obtain the model of the activated enzyme (i.e. containing the reactive oxoferryl moiety, responsible for substrate oxidation) the reaction with molecular oxygen was modelled using QM/MM methods. In parallel a set of auxiliary simplified models for the active site was prepared with the purpose of elucidating their inherent redox and acidobasic properties (redox potentials and acidobasic constants, respectively) and the mechanisms for the hydrogen abstraction reaction (with the set of auxiliary substrates). The correlation of the inherent properties and the reaction mechanism will allow to elucidate to what extent redox and acidobasic properties of the active site of the studied enzyme shape its activity.

## **Synthesis of new conjugates of neuroleptics belonging to phenothiazine derivatives with gold nanoparticles and evaluation of their activity in protein fibrillation processes**

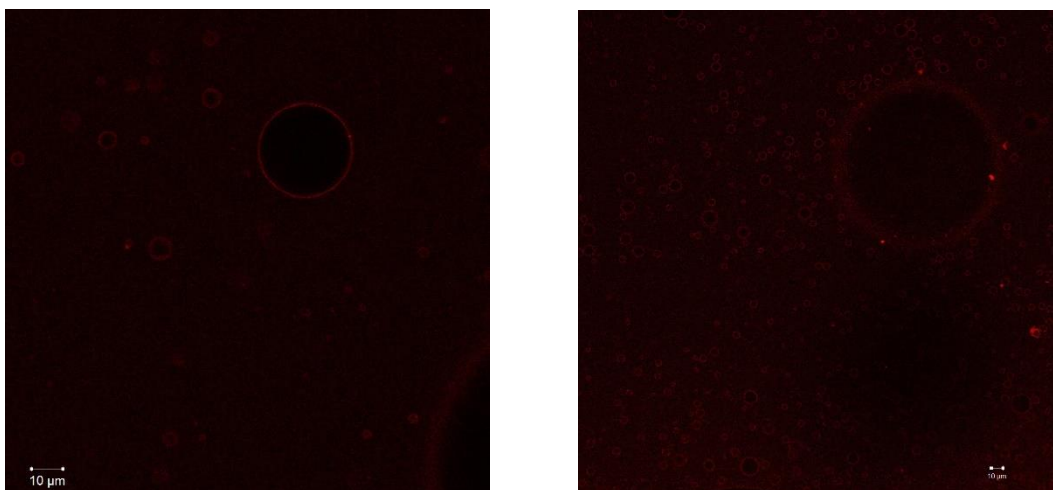
"Miniatura" research project NCN 2022/06/X/ST5/00350 [2022-2023]  
(Project leader: Magdalena Oćwieja DSc)

Two types of new conjugates of neuroleptics (phenothiazine (PTZ) derivatives) with gold nanoparticles (AuNPs:  $15\pm 6$  nm  $55\pm 3$  nm) were obtained via the ligand exchange reaction of citrate anions on the surface of AuNPs. This process was conducted in the presence of chloride anions. In the experimental works the attention was focused on the preparation of conjugates of chlorpromazine (CPZ) and fluphenazine (FPZ). The results of electrokinetic investigations showed that the zeta potential of PTZ-AuNP conjugates strongly depends on the quantity of adsorbed PTZ molecules. The PTZ-AuNP conjugates characterized by positive surface charge ( $50\pm 8$  mV for pH 5.8 and ionic strength  $1 \text{ mol/dm}^3$ ) were obtained after the total saturation of AuNP surface by PTZ molecules. The destabilization of PTZ-AuNP conjugates was observed at ionic strength higher than  $10 \text{ mol/dm}^3$  which was detected using UVvis spectroscopy and dynamic light scattering technique (DLS). The results of studies obtained using electrophoretic light scattering technique (ELS) revealed that the zeta potential of conjugates decreases with pH increase (e.g., for CPZ-AuNPs  $57\pm 3$  mV and  $-50\pm 2$  mV for pH 4,0 and 10,1, respectively). CPZ-AuNPs exhibited an isoelectric point at pH 8.8 whereas FPZ-AuNPs at pH 8.5. The spectroscopic investigation, including UVvis and surface enhanced Raman spectroscopy (SERS), enabled the detection of adsorption structure of both drugs on the surface of AuNPs. The results of UVvis measurements revealed the destabilization of conjugates under alkaline conditions. Moreover, it was found that the conjugates dispersed in the DMEM culture medium supplemented by FBS remained stable at a temperature of  $37^\circ\text{C}$  for 28-30 h. Based on the data obtained from the MTT assay, it was determined that the application of CPZ and FPZ at a concentration of  $10^{-3} \text{ mol/dm}^3$  reduced the viability of SH-SY5Y cells to 64-73%. AuNPs stabilized with citrate anions at a concentration of  $50 \text{ mg L}^{-1}$  did not reduce cell viability. Therefore, this concentration range was chosen for the assessment of cytotoxicity of the conjugates. The cytotoxicity studies of CPZ-AuNPs and FPZ-AuNPs conjugates (characterized by diverse compositions: PTZ at concentrations of  $10^{-6}$ ,  $5\times 10^{-5}$  and  $10^{-4} \text{ mol/dm}^3$ ) revealed that the cytotoxicity of the conjugates was significantly higher than that of AuNPs. Moreover, FPZ-AuNPs conjugates were less toxic than CPZ-AuNPs. Conjugates composed of  $20 \text{ mg/L AuNPs/ } 10^{-6} \text{ mol/dm}^3$  PTZ were selected for fibrillation studies. The results of protein adsorption studies on monolayer surfaces of AuNPs and PTZ-AuNPs demonstrated that the process is effective at both pH 7.4 and 11. However, under alkaline conditions, the amount of adsorbed protein was considerably lower than under neutral conditions. This confirms the predominant role of electrostatic interactions in the processes of protein monolayer formation. It also highlights the influence of the heterogeneous distribution of protein charge on the adsorption occurring for particles characterized by the same charge as the layers of AuNPs and PTZ-AuNPs. Fibrillation kinetics measurements, conducted using the ThT test in the presence of PTZ, trisodium citrate, and AuNPs, did not indicate any pro-fibrillar properties of the substances at pH 7.4 and a temperature of  $37^\circ\text{C}$ . At pH 7.4 and 11.0, CPZ and FPZ most effectively inhibited fibril formation. The AuNPs slightly hindered fibril formation, but the effect became noticeable at an elevated temperature ( $60^\circ\text{C}$ ) after an incubation period exceeding 350 hours. No significant differences were observed in inhibiting the fibrillation process by the AuNPs and PTZ-AuNP conjugates. Microscopic studies revealed that fibrils formed in the presence of CTZ-AuNPs conjugates exhibited significantly shorter lengths compared to control fibrils.

## Synthesis of new conjugates of neuroleptics belonging to phenothiazine derivatives with gold nanoparticles and evaluation of their activity in protein fibrillation processes

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

As part of the task carried out since the end of November 2023, I have purchased the necessary laboratory equipment and reagents required for the synthesis of copolymers and target emulsions. I have conducted conceptual and organizational work, and access to laboratory 025 has been arranged for proceeding the RAFT polymerization of co-polymers. As part of the conceptual work, images of emulsions stabilized with BLG microgels were also captured using confocal microscopy, stained with Fast Green dye, providing a proper method for thermo-sensitive emulsions envisaged in this project. The results are attached below in Fig. 1.



## **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 main objective of this research project is to investigate potential application of *bio*-polyelectrolytes (poly-L-arginine, poly-L-lysine, polyglutamic acid, sulfated polysaccharides) and various, including *bio*-, surfactants (quaternary ammonium surfactants, rhamnolipids, ethyl lauroyl arginate) in stabilization of foam films formed under static and dynamic conditions. The choice of polyelectrolyte/surfactant system components is driven by their properties, like: biodegradability, biological activity (antiseptic), and biocompatibility. Those properties are interesting in terms of further development of reliable technologies for producing foam for application in decontamination of chemical, biological, and nuclear agents. Using complementary approach, i.e. application of experimental techniques allowing to study foam films under static and dynamic conditions, the synergetic and antagonistic effects of mixed polyelectrolyte/surfactant systems in foam films stability will be thoroughly and systematically examined. Results obtained within this project framework will be used for verification of the following hypotheses: (i) length of the PE chain and its conformation and (ii) dynamic adsorption layer formed at the rising bubble surface are crucial for kinetics of the foam films drainage.

Surface tension measurements of the polyelectrolyte/surface active substance mixtures have been started. Preliminary results about influence of polyelectrolyte/surface active substance mixtures on bubble velocity in solutions have been obtained. Moreover, physicochemical characteristic of polyglutamic acid (PGA) is in the progress, including: measurements of viscosity and density of polyelectrolyte solution to determine structure and specific density of PGA, measurement of electrokinetic potential of PGA.

## **Stability and physicochemical properties of foams formed from solutions of surface active substances and polyelectrolytes**

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

As part of the work carried out in 2023, a device that allows carrying out strong surface plastic deformation based on the principle of surface mechanical abrasive machining (SMAT) was designed and constructed. The SMAT method involves plastic deformation of the machined surface with repeated, high-energy impacts with a spherical abrasive with a diameter of several millimeters. In the proposed solution, the sample is placed stationary in a horizontal position in a chamber, the base of which vibrates at a high frequency, setting the abrasive in motion. Multidirectional impacts fragment the grains of the processed metal to a nanometer scale. The constructed device is based on a commercial vibration generator system.

The literature review showed that the biggest problem during surface deformation methods based on peening is the contamination of samples with iron coming from the abrasive material and, as in the case of the SMAT method, from the chamber itself in which the process is carried out. Therefore, steel elements are eliminated in the constructed system. The chamber is made of chemically inert material and its base is made of commercially pure Mg. To carry out plastic deformation on magnesium samples, a much harder material than steel was used - balls made of ZrO<sub>2</sub>.

SMAT deformation tests were carried out on Mg samples with a purity of min. 99.95%. Balls made of ZrO<sub>2</sub> with diameters of 2 mm and 4 mm were used, the process was carried out for 1 hour, the vibration amplitude of the chamber base was 50 Hz. Electrochemical tests in NaCl solutions of the samples prepared in this way were started. Measurement systems were built to collect the released hydrogen only from the surface that was subjected to the SMAT process. Comparative tests of samples subjected to surface deformation and covered with conversion layers with samples not subjected to SMAT and covered with conversion layers were started.





**Research Projects  
of the National Centre for Research and Development**



## **Outperforming functionality: composite/mixed matrix porous materials in membrane-based processes**

„EIG CONCERT-JAPAN” research project NCBiR EIG CONCERT-JAPAN/1/2019 [2019-2023]  
(Principle investigator: Aleksandra Pacuła D.Sc. Eng.)

The aim of the study was the synthesis and characterization of carbon materials dedicated for the production of polymer composite membranes. The research also included the determination of the electrical conductivity and dielectric constant of membranes. The carbons were synthesized at 600 and 700 °C via acetonitrile pyrolysis, in the presence of a porous substrate consisting of a mixture of metal oxides generated in-situ as a result of thermal decomposition of layered double hydroxides (LDHs) of different chemical composition (MgMnAl, MgFeAl, MgCuAl, NiAl). Porous, N-doped carbons differing in the morphology of carbon grains (lamellar and tubular), specific surface area (various contributions of micro- and mesopores) were obtained. The carbon materials were made of turbostratic carbon with structural defects – showing a structural arrangement similar to graphite, but with an interlayer distance greater than that observed in graphite, and a certain amount of amorphous carbon. Polymer membranes were prepared with the use of a biocompatible polymer – polycaprolactone (PCL) using two methods. The 1<sup>st</sup> method involved the preparation of a PCL suspension in a liquid with low volatility (N-methylpyrrolidone (NMP), boiling point 202 – 204 °C) and PCL concentration = 15 % (method I), followed by spreading the PCL suspension on the surface of a glass plate (15 cm by 20 cm) with an applicator adjusting the thickness of 200 μm and separating the membranes in a coagulation bath (isopropanol). The 2<sup>nd</sup> method consisted of the preparation of a PCL suspension in a liquid with high volatility (dichloromethane (DCM), boiling point 39.6 °C) and PCL concentration = 5 % (method II), followed by distribution of the PCL suspension at the bottom of Petri dish ( $\phi = 9,4$  cm) and DCM evaporation. In both cases, membranes with a thickness of 100 μm were obtained. Due to the limitations resulting from the durability of the membranes, the addition of carbon in PCL membranes prepared by method I was small and equal to 0.7 wt. %, while the addition of carbon in PCL membranes prepared by method II was higher (2.4 – 4.6 wt. %). The electrical conductivity and the dielectric constant of PCL membranes were determined by measuring the electrical resistance and the electrical capacitance, respectively. The measurements were made with the LRC meter using different frequencies ( $\nu = 100$  Hz – 1 MHz) of alternating current, using a parallel equivalent circuit to describe the tested system. The membrane was placed between two brass cylinders of the same diameter (ca. 4 cm), on the side of the polished and cleaned surface (the contact area of the brass disc with the membrane was 12.2 cm<sup>2</sup>). The electrical conductivity and the dielectric constant ( $\epsilon$ ) of PCL membranes for  $\nu = 1$  kHz was  $2.4 - 3.0 \times 10^{-10}$  S m<sup>-1</sup> and 1.2 – 1.9, respectively. Small addition of carbon (0.7 % wt.) did not modify the electrical conductivity and  $\epsilon$  value of the composite membranes. Only higher addition of carbon using method II affected  $\epsilon$  (2.2 – 9.14) and allowed to obtain conductive composite membranes. It turned out that lamellar grains contributed less to the improvement of conductivity. The highest conductivity of  $5.4 \times 10^{-4}$  S m<sup>-1</sup> was shown by the composite membrane obtained with the addition of 4.6 wt. % carbon, in which carbon nanotubes dominated.

## **Technology of biorefining vegetable oils for the production of advanced composite materials**

„TechMatStrateg” research project NCBiR TECHMATSTRATEG2/407507/1/NCBR/2019 [2019-2022]

*(Project leader: Associate professor Maciej Guzik)*

The primary goal of the project was to create a demonstration line - a biorefinery module for the production of a unique family of bacterial polymers - polyhydroxyalkanoates (PHA). This goal was achieved - at the end of 2022, the prototype building of the Biorefinery at Niezapominajek 8 in Krakow was completed, where the entire prototype line was moved from rented premises at Balicka 100 in Krakow. The entire prototype line underwent validation and achieved satisfactory parameters in 2023. The outfitting of the prototype's casing with necessary installations (e.g., a container, a technological gas line) was completed.

The second goal of the project was to demonstrate the potential of PHA polymers (including PHB) in the development of the Polish economy, as exemplified by the preparation of composite materials for spatial forming (e.g., bone tissue implants or other advanced post-implant composite forms (flexible materials used for industry – textiles, food packaging, toys, and other plastic products). By December 2021, as part of the Consortium, semi-products for composites were produced and characterized, namely: ceramic powders, three-dimensional, high-porosity ceramic sponges, and produced polymers PHB and aPHA. In subsequent steps, polymer composites (PHB and aPHA blends) and ceramic-polymer composites (infiltrated porous ceramic scaffolds covered with polymers (PHB, mcl-PHA)/PHA blends, as well as extruded polymer filaments/blends PHA containing ceramic powders) were obtained from these materials. These materials were subjected to extensive physicochemical and mechanical characterization, and in vitro biological characterization. Studies were conducted to evaluate selected materials in a rabbit in vivo model. Simultaneously, in the Consortium, we were able to preliminarily demonstrate the usefulness of the produced materials in post-implant applications (e.g., 3D printing).

## **CULDIGI - Integrated digitalization, filing and documentation system for cultural heritage (images and 2D artefacts)**

„Intelligent Development Operational Programme” research project NCBiR POIR.04.01.04-00-0145/19 [2021-2023]

*(Project leader: Associate professor Jakub Barbasz, Leszek Krzemień PhD)*

As part of the implementation of the project for an integrated system for the digitisation, cataloguing and documentation of cultural heritage (images and 2D artefacts), all the work envisaged in the proposal was completed in 2023.

A scanning device has been built, enabling the collection of image data with high resolution, comparable to optical microscopes (magnifications typically from 0.5x to 4x). It is also possible to work at higher magnifications when using optional lenses (microscope objectives). This makes it possible to obtain images with a resolution of 1 micrometre per 2 pixels and a scanning area size of more than 50 by 50 cm<sup>2</sup>.

The images obtained can be enhanced with additional channels containing information such as a 3D image collected from a laser sensor. They can also be scanned using near-infrared and ultraviolet light.

The project has achieved results that significantly exceed the parameters (especially scanning speed) of commercially available systems.

The developed solution can be used for detailed and high-resolution archiving of works of art, scientific research and digital access to all 2D and pseudo 2D objects (with little texture - such as oil paintings, parchments or copper engraving plates).

The developed device also allows working with stroboscopic light synchronised by hardware with the camera shutter. As a result, it is possible to reduce the exposure of valuable works of art to visible, infrared and ultraviolet radiation to a level of 0.1-1% of the dose with which commercially available solutions work. It is possible to scan and archive light-sensitive artefacts.

## **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*)

In the current reporting period, microemulsions, namely PHN-glu, PHN-gal, PHN-lac and F-PHN-glu, F-PHN-gal, F-PHN-lac, were produced using the obtained sugar esters (SFAE). Physicochemical characterization of these emulsions was carried out using dynamic light scattering (DLS). This was followed by *in vitro* studies using MTT survival assay on selected colon cancer cell lines (lines: HCT116, SW480, Caco2, Colo205), as well as on non-cancer control lines (HEK293, BJ). In the current year, *in vitro* studies were repeated using ECKT alone, and both emulsions alone and those containing the chemotherapeutic agent SN-38 were tested. It was found that PHN fluorine-modified lactose (F-PHN-lac) and galactose (F-PHN-gal) esters, as well as unmodified lactose esters (PHN-lac), were the most toxic against cancer lines. The range of effective concentrations of SFAE was 0.125-0.5 mg/ml, respectively. Emulsions containing no chemotherapeutic agent showed no cytotoxicity. The chemotherapeutic agent in the emulsions did not show increased and more selective cytotoxicity compared to the chemotherapeutic agent alone. However, two cell lines were identified that responded best to it: SW480 and HCT116.

In order to increase the therapeutic potential of ECKT and emulsions based on them, a new method was developed to produce emulsions based on PHN-lac and fluorine-modified F-PHN monomers. The emulsions thus prepared, containing SN-38, were also subjected to MTT tests on SW480 and HCT116 lines. After statistical analyses, the newly produced emulsion was found to significantly support the action of SN-38, qualifying it for the next stage of the project - *in vivo* testing.

Working with the Maria Skłodowska-Curie National Institute of Oncology in Warsaw, an application was drafted to the ethics committee to obtain approval for experiments on mice with implanted HCT116 cells. In parallel, both during and after the *in vitro* stage, a detailed market analysis was carried out in the context of applications and the possibility of implementing both the esters and the newly produced emulsions in the medical and pharmaceutical industries. The analysis was concluded with a report and an expert interview, in which specialists from the National Cancer Institute, working with biomedical companies, answered the survey questions.

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





## **International Scholarship Exchange of PhD Candidates and Academic Staff**



Project NAWA PPI/PRO/2018/1/00006/U/001 PROM [2019-2023]

*(Project coordinator: Professor Tomasz Borowski)*

In December 2022 a competition was opened for scholarships to finance short-term internships in foreign research units for doctoral students of ICSC PAS. This competition was resolved in January 2023.

During 2023 the following short forms of education were realized within the PROM Program:

- two female PhD students from IKiFP PAN took part in a scientific conference abroad;
- two female and two male PhD students from IKiFP PAN completed a research internship at a foreign institution;
- one male PhD student from abroad completed a research internship at IKiFP PAN;
- one female research staff member from abroad completed a research internship at IKiFP PAN.

## Structure and Function of Protein Corona at the Nanoparticles Interface



Project NAWA Canaletto PPN/BIT/2021/1/00089/U/00001 [2022-2023]

(Project leader: Professor Barbara Jachimska; co-investigator: Agnieszka Kamińska PhD)

Drug delivery systems (DDS) are of great importance due to their ability to overcome biological barriers and reach the target directly, avoiding side effects. However, the behavior of carriers in contact with biological fluids is determined by the "protein corona effect". Proteins adsorbed on the DDS surface thus change their behavior, physicochemical properties, stability, cellular uptake, endocytosis, and pharmacokinetics. Depending on the affinity for the nanoparticle surface, the protein corona can be classified as "hard corona" and "soft corona". The hard corona is formed by irreversibly bound proteins, while the soft corona is formed by low-affinity proteins that have a reversible interaction with the surface of the nanoparticles. Moreover, the proteins forming the protein corona can be divided into opsonins and dysopsonins. The first group promotes the rapid removal of nanoparticles from the bloodstream, while the second group prolongs their circulation. By controlling the binding of dysopsonins to nanoparticles, the bioavailability of nano-based drug delivery systems can be increased. To achieve this and understand the fate of nanoparticles under biological conditions, it is necessary to investigate how to design efficient adsorption of dysopsonin onto the surface of nanoparticles.

Mesoporous silica nanoparticles (MSN) with a size in the range of 50-300 nm and a large specific surface area (300-1500 m<sup>2</sup>/g) were selected for testing. The transport of nanocarriers in vivo is influenced by properties such as size, shape, degree of hydrophobicity, and charge. MSNs are characterized by good thermal and chemical stability, ease of functionalization, and the ability to accumulate a large number of active particles inside the porous structure. The type of silica used in the research was MCM-41. Selected silica structures have pores in the form of channels arranged parallel to each other and forming a hexagonal phase. To obtain a complete characterization of the system, the physicochemical properties of MSNs in the presence of BSA were investigated using a wide range of physicochemical methods such as QCM-D, CD, FT-IR, UV-vis, contact angle, DLS, and zeta potential.

# **Horizon Programme**



## **IPERION HS - Integrating platforms for the European Research Infrastructure on heritage science**

Horizon 2020 Programme no. 871034 [2020-2024]

*(Project leader: Professor Łukasz Bratasz, Marcin Strojecki PhD, Marcin Bury PhD, Łukasz Berger Eng.)*

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.

## 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@shell" 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<sub>3</sub>, 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.

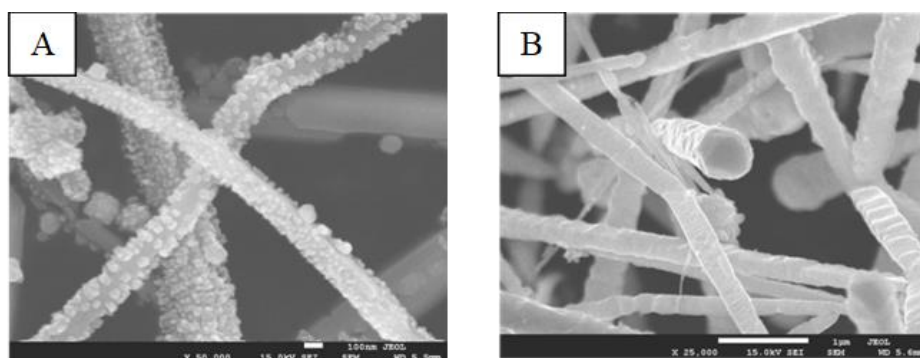


Fig 1. SEM images of Cu@Ag NWs in the presence of ascorbic acid, which was added immediately (A) and after 3 minutes (B) of adding AgNO<sub>3</sub>.

## NewCat - Teaching Lytic Polysaccharide Monooxygenases to do Cytochrome P450 Catalysis



Horizon Europe EIC-PATHFINDER nr 101046815 [2022-2025]

*(Project leader: Professor Tomasz Borowski, Anna Miłaczewska-Kręgiel PhD, Anna Kluza PhD, Zuzanna Wojdyła PhD)*

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 consortium 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).

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## **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, investigators: Łukasz Berger, Sonia Bujok PhD Eng., Marcin Strojecki PhD, Marcin Bury PhD, Magdalena Soboń, Nefeli Avgerou)*

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  $88 \pm 6$  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 °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.



## **EU COST Actions**



## Computational materials sciences for efficient water splitting with nanocrystals from abundant elements



EU COST Action no 18234 [2019-2023]

(country coordinator: Professor Małgorzata Witko)

This COST Action intends to focus on bridging the knowledge gaps between different theoretical methods (DFT, molecular mechanics and molecular dynamics, Monte-Carlo dynamics, microkinetic modelling) and computer codes in order to facilitate the modelling of all processes which accompany water splitting, in a longer term enabling the discovery of novel electrocatalytic materials. The calculations done at the ICSC PAS are employing Density Functional Theory (DFT) method, with PBE functional and def2-TZVP basis set.

This year, the possibility of using  $V_2O_5$  as a catalyst in the electrocatalytic water decomposition reaction was investigated. For this purpose, a two-layer cluster model of the (010) surface of vanadium pentoxide of the formula  $V_{20}O_{62}H_{22}$  was constructed. This cluster reflects the structure of the represented surface, on which three types of oxygen atoms can be distinguished (so-called vanadyl, coordinated with a single vanadium atom - O1, bridging, coordinated with two vanadiums - O2, and triple-coordinated with vanadium - O3) of different nucleophilicities. Since it is assumed that the water decomposition reaction takes place on the defective surface of oxide catalysts, models of the  $V_2O_5$  cluster were constructed with oxygen vacancies present at the positions of various oxygen atoms (O1, O2, O3). The ease of creating single oxygen vacancies varies in the series  $O1 < O3 < O2$ . In the next step, it was found that in the case of the formation of oxygen vacancy pairs, the formation of vacancy groups of the same type is more favorable. The formation of oxygen vacancies is accompanied by a change in the properties of vanadium pentoxide, which changes from a semiconductor to a metallic system (the band gap is reduced by 96% - 100% compared to a system without defects).

Vacancies are the places where water molecules bind.  $H_2O$  is bound the weakest in the O1 position and the strongest in the O3 position. It was also found that both undefected  $V_2O_5$  and  $V_2O_5$  with introduced oxygen vacancies are able to dissociatively adsorb the  $H_2O$  molecule. Dissociative water adsorption on the (010) surface is possible in various configurations. The proton resulting from dissociation can form an OH group with oxygen O1, O2 or O3, and the OH group formed as a result of dissociation can attach to vanadium that already has a vanadyl group ( $V=O$ ) to form the  $HO-V=O$  moiety or can adsorb on the surface free V.

The calculation results showed that in the first case, the  $HO-V=O$  group on the surface and the OH group (with the participation of the O3 oxygen) are formed. The OH group thus created is directed deep into the cluster, between the layers, which is the first step towards the formation of vanadium bronzes. When the OH group formed as a result of dissociation adsorbs on "bare" vanadium and  $H^+$  on O1 or O3, water is regenerated and stays physisorbed on the surface (at a distance over 2.5 Å above the surface).

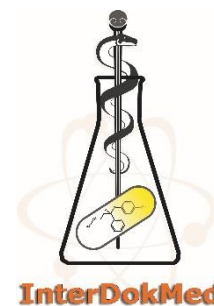


## **Projects of EU Structural Funds Programmes**



## Cross-institutional PhD Studies „Interdisciplinarity for Innovative Medicine” InterDokMed

EU Structural Funds project NCBR POWR.03.02.00-00.I013/16 [2017-2023]  
(project leader: Associate Professor Renata Tokarz-Sobieraj)



The aim of the project was to implement interdisciplinary doctoral theses in the field situated at the intersection of exact sciences and medical sciences. The proposed doctoral study program aligned with the trend of modern education. The doctoral studies had an environmental character, and the selection of partner units guaranteed a high level of the developed education model. The project was carried out by a consortium composed of five scientific units: the project coordinator was the Jerzy Haber Institute of Catalysis and Surface Chemistry PAS and the partners: Jerzy Maj Institute of Pharmacology PAS, Henryk Niewodniczański Institute of Nuclear Physics PAS, the Faculty of Chemistry and the Faculty of Medicine of Jagiellonian University.

The interdisciplinary nature of the doctoral program was ensured through: (1) research topics carried out in partner units, encompassing more than one discipline (chemical, physical, medical, pharmaceutical sciences), (2) execution of doctoral theses under the guidance of two supervisors representing different disciplines and originating from two different partner units, making the project multidisciplinary, (3) mandatory seminars common to all doctoral students, serving as a forum for exploring new, innovative methodological solutions that connect and complement specialized research methods from various scientific disciplines, making the project transdisciplinary, (4) addressing issues and applying methods, tools/objects of research typical of one field in others, giving the project characteristics of cross-disciplinarity. PhD students had the opportunity for internships abroad and collaboration with international partners.

The thematic of the doctoral dissertations focus aligned with issues crucial to the economy and society. These topics supported the innovation of the country and provided opportunities for the transfer/commercialization of doctoral study results. The themes, situated at the intersection of exact and medical sciences, concentrated on the exploration of new materials for medical or pharmacological applications, investigations into the mechanisms of action of existing drugs using modern physicochemical techniques applied in exact sciences, and the understanding of potential molecular mechanisms of protein action in disease processes. The foundation of these works included complementary model studies and research on experimental animals, as well as clinical material obtained from patients. This approach allowed for a comprehensive exploration of the identified topics, combining model research, experiments on animals, and clinical material obtained from patients to gain a deeper understanding of the issues at the intersection of exact and medical sciences.

44 PhD students successfully defended their doctoral dissertations and obtained the doctoral degree: 16 in the field of exact and natural sciences with a focus on chemical disciplines, 9 in the field of exact and natural sciences with a focus on physical disciplines, and 17 in the field of medical sciences and health sciences with a focus on medical disciplines. Additionally, 1 individual earned their doctorate in the field of medical sciences and health sciences with a focus on pharmaceutical disciplines. A total of 18 interdisciplinary doctoral theses were distinguished. As part of the project, 275 publications, 14 book chapters, and 39 popular science and post-conference publications were produced. Doctoral candidates were co-authors of 240 conference presentations and 372 posters.

The project co-financed by the European Union through the European Social Fund was carried out within the framework of the Operational Program Knowledge Education Development 2014-2020, Action 3.2 Doctoral Studies, Axis III Higher Education for the Economy and Development. The project's goal was to enhance the quality and effectiveness of education in doctoral studies.



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## **Interdisciplinary Environmental Doctoral Studies "Physical, Chemical and Biophysical Foundations of Modern Technologies and Materials Engineering" (FCB)**

Project of Operational Program Knowledge Education Development (POWER) for interdisciplinary doctoral study programs POWR.03.02.00-00-I004/16 [2017-2023]

(*deputy project manager: Anna Micek-Ilnicka DSc. Eng.*)

The FCB project was completed according to scheduled date (31st October 2023). This project was financed by the Foundation for the Development of the Education System and was a result of a competition announced by The National Centre for Research and Development (NCBiR) in 2017 for interdisciplinary doctoral studies programs. This FCB project was coordinated by the Faculty of Physics and Applied Computer Science (WFiIS) at the AGH University of Science and Technology (AGH) with the participation of four institutions; the Faculty of Materials Science and Ceramics (WIMiC) at the AGH, the Faculty of Chemistry at Jagiellonian University (WCh UJ), The Henryk Niewodniczański Institute of Nuclear Physics (IFJ) of the Polish Academy of Sciences and Jerzy Haber Institute of Catalysis and Surface Chemistry of the Polish Academy of Sciences (IKiFP).

The FCB studies were interdisciplinary. PhD students represented institutions conducting research in the field of disciplines such as chemical and physical sciences as well as in the field of engineering and technical sciences in disciplines such as materials engineering and chemical engineering. The scientific achievements of PhD students from IKiFP (3 people) during FCB studies include: 19 publications, 1 international patent and 2 Polish patents, 30 oral presentations and 21 poster presentations during the conferences and congresses

All project factors declared in the project were achieved. The project assumed the education of 75 PhD students, and in the final result, 72 doctoral students completed FCB studies. Other project factors such as: the number of completed doctoral theses was 62 (per 60 planned theses) and the number of doctoral theses with two supervisors was 40 (per 40 planned theses). As part of FCB project 3 PhD students from IKiFP completed the promotion procedures with obtaining a doctoral degree, which was agreed upon with the project assumptions.



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## **Other International Research Projects**



## **Bioplastics upcycling loop (BioPolyCycle)**

ANSO-CR-PP-2022-01

*(project leaders: Maciej Guzik DSc)*

The study introduces an innovative upcycling method for transforming commercial bioplastics like polylactide (PLA), polycaprolactone (PCL), and polyhydroxyalkanoates (PHA) into valuable carbon-rich feedstocks. These feedstocks are utilized for biotechnological production of biopolymers such as PHA and bacterial cellulose (BC). This approach is strategically aligned with nine United Nations' Sustainable Development Goals, contributing to improved health, efficient waste management, clean water, sanitation, and sustainable industrial practices.

Key aspects of the methodology included developing fermentation techniques using carbon-rich feedstocks, primarily focusing on microbial conversion to PHAs. Initial experiments in flasks assessed various bacterial strains for PHA biopolymer production, identifying hydroxylated fatty acids as potential substrates, despite their mild toxicity. Specific pulse feeding strategies were crafted for these compounds. Subsequent batch fermentations established essential fermentation parameters, leading to optimized feeding strategies to enhance cell density and PHA content. The extraction of PHA polymers was performed using solvent processing at various temperatures.

Initial findings are promising, demonstrating the feasibility of producing PHA polymers with various bacterial strains and hydroxylated fatty acids as substrates. Despite the substrates' slight toxicity, effective pulse feeding strategies were developed. These preliminary results, obtained from initial batch fermentations, provide vital data for future research and highlight the potential of these substrates in generating diverse PHA polymers.

## Project “Learning mobility of individuals”



EU Erasmus+ Programme [2014-2023]  
(*coordinator of the project: Agata Pomorska PhD.*)

In 2023, the following learning mobilities took place:

- a) students for traineeships
  - Amir Sultan – St Andrews University (Szkocja)
  - Zofia Krasieńska-Krawet – University of Zagreb (Chorwacja)
  
- b) staff members for training
  - Andrzej Baliś – Laboratoire Charles Coulomb (France)
  - Marcin Bury – Norwegian University of Science and Technology (Norway)
  - Dzmitry Kharytonau – Helmholtz- Zentrum Hereon (Germany)
  - Marcel Krzan – Sofia University "St. Kliment Ohridski" (Bulgaria)
  - Marcel Krzan – Institute of Condensed Matter Chemistry and Technologies for Energy, National Research Council (Italy)
  - Leszek Krzemień – Universität Erlangen-Nürnberg (Germany)
  - Aneta Michna – Clausthal University of Technology (Germany)
  - Aneta Michna – University of Ostrava (The Czech Republic)
  - Maria Morgia – Clausthal University of Technology (Germany)
  - Maria Morgia – University of Ostrava (The Czech Republic)
  - Aleksandra Pacuła – Universidad de Cantabria (Spain)
  - Agata Pomorska – Clausthal University of Technology (Germany)
  - Agata Pomorska – University of Ostrava (The Czech Republic)
  - Małgorzata Ruggiero – Institute of Chemical Engineering Sciences, FORTH/ICE-HT (Greece)
  - Konrad Skowron – Helmholtz- Zentrum Hereon (Germany)
  - Mateusz Tataruch – Slovak University of Technology in Bratislava (Slovakia)

The participation in the mobilities resulted in the collection of research data for PhD thesis, articles in scientific journals of international scope and for conference contributions.

The project will continue in 2024.

