BIOPHYSICS

Understanding the structure-stability-cellular internalization relationship of polymeric nanoparticles for targeted drug transport.
supervisor: prof. RNDr. Pavol Miškovský, DrSc. (pavol.miskovsky@upjs.sk)
consultant: Shubhashis Datta, PhD
study form: full time
Annotation: Nanoparticles formed by the self-assembly of amphiphilic copolymers have gained increased attention as a smart carrier for the improved solubilization and efficient delivery of anticancer agents. Many of those nanoparticles have entered clinical trials and some are in clinic use. To perform high therapeutic efficacy after an intravenous injection of polymeric nano-drug formulation, its stability during circulation in blood compartments is prerequisite for drug delivery. The aim of this thesis is to study and understand the behavior of polymeric nanoparticles under conditions *in vivo* such as extreme dilutions and interactions with blood proteins (e.g. serum albumin, globulin) and cells. The PhD student will investigate how minor structural changes of copolymer will affect the stability-activity relationship of nanoparticles which may play crucial role in designing effective polymeric nanoparticles for biomedical applications.

Development of nanosensors based on plasmonic-enhanced optical spectroscopy for sensitive and selective detection of SARS-Cov-2 mutants).
supervisor: prof. RNDr. Pavol Miškovský, DrSc. (pavol.miskovsky@upjs.sk)
consultant: prof. Santiago Sanchez-Cortes, PhD
study form: full time / co-tutorring (UPJŠ, Universita Autonoma de Madrid)
Annotation: The interaction of light with nanostructures of surface of metals, such as silver or gold, produces a large intensification of electric field on the metal surface. This is the basis of the so-called Plasmonic-Enhanced Optical Spectroscopy - PEOS (Raman, Fluorescence and IR) that leads to a huge enhancement of spectroscopic signal from molecules placed on the metal surface. PEOS techniques are based on nanotechnology and in the last 20 years a boom in the applications of PEOS have been noted due to the development of new and magic nanostructured metal substrates able to induce a high intensification of the electric field. In this PhD work, the fabrication of new and feasible nanostructures based on plasmonic metals is intended. These new nanosensors will be prepared under specific architectures and morphologies in order to find surfaces with innovative properties to be applied in the detection of a large list of pollutants (environment), biomolecules (medicine). colorants of interest (cultural heritage) and molecules of interest in industry (chemical, pharmaceutical).
Remark: The subject of the thesis is based on the development of breakthrough PickMol™ technology by SAFTRA photonics. In the period of the doctoral study the student will have an unique occasion to work with leading global companies in environment, chemistry and food industry.

Development of a nano-transport system for drug delivery and bioimaging.
supervisor: RNDr. Veronika Huntošová, PhD. (veronika.huntosova@upjs.sk)
study form: full time
Annotation: Targeted therapy is one of the most promising approaches for tracing cancer cells and neutralizing them non-invasively. Several approaches have been developed in this area to increase targeting effectiveness. The doctoral student's task will be to propose an approach on how to effectively target diseased tissue through a

biocompatible nanoparticle system. The delivery system will be designed not only to deliver drugs, but also to play an active role in bioimaging. When performing tasks within the dissertation project, the student will use the methods of fluorescence spectroscopy, microscopy, bioimaging. The methods of flow cytometry, immunolabeling, western blot and PCR will be used to study the effectiveness of the system. The study will be performed in 2D and 3D cell cultures and a preclinical model of the avian chorioallantoic membrane. Within the project, the student will actively cooperate with other laboratories in Slovakia and abroad.

Investigation of the signalling pathways active in a photobiomodulation and photodynamic therapy.
supervisor: RNDr. Veronika Huntošová, PhD. (veronika.huntosova@upjs.sk)
study form: full time
Annotation: The application of light plays an important role in the process of regeneration, photodiagnostics and treatment of cancer. It is often a non-invasive form of therapy that is well tolerated by patients. Depending on the wavelength of the radiation source used, the light dose and the concentration of the drug, it is possible to achieve cell regeneration as well as cell death. The role of the doctoral student will be to identify the mechanism of action of photobiomodulation and photodynamic therapy with respect to the endomembrane system, cell metabolism and signalling molecules leading to cell regeneration or death. The student will use the methods of fluorescence spectroscopy, microscopy, bioimaging, flow cytometry, immunolabeling, western blot and PCR to perform the tasks within the dissertation project. The cell cultures in 2D and 3D, and a preclinical model of the avian chorioallantoic membrane will be used in the study. Within the project, the student will actively cooperate with other laboratories in Slovakia and abroad.

Purification and characterization of selected variants of staphylokinase.
supervisor: doc. RNDr. Erik Sedlák, DrSc. (erik.sedlak@upjs.sk)
consultant: Mgr. Mária Tomková, PhD.
study form: full time
Annotation: Bacterial staphylokinase (SAK) has been studied as a promising third-generationof trombolytic drugs in the treatment for vascular occlusion. To date, SAK was successfully tested in number of clinical trials and there are also some ongoing clinical studies. Mature SAK is a 136 amino acid (15.5 kDa) single chain extracellular protein secreted by lysogenic strains *of Staphylococcus aureus*. In humans, SAK initiates the fibrinolytic cascade to help invading bacterium move deeper into the tissues, therefore SAK has been studied as a thrombolytic drug. The aim of the dissertation is to provide a detailed analysis and characterization of biophysical and biochemical properties of individual staphylokinase mutants obtained by directed evolution of proteins with an emphasis on the study of their stability and affinity to plasmin.

Development of efficient genetically encoded photosensitizers based on flavoproteins.
supervisor: doc. RNDr. Erik Sedlák, DrSc. (erik.sedlak@upjs.sk)
consultant: doc. Mgr. Gregor Bánó PhD.
study form: full time

Annotation: Traditional small molecule drugs and photosensitizers (PS) used in photodynamic therapy lack selectivity towards diseased tissues and are known to distribute also throughout healthy tissues. The alternative to relatively nonspecific targeting of nanoparticles is utilization of the proteins containing fluorescent PS that represent genetically encoded singlet oxygen generators, which can be, in principle, targeted to diseased biological tissues. In this project, we propose fundamentally different approach to design efficient genetically encoded singlet oxygen generators based on flavoproteins This approach relies on identification of suitable positions of amino acids nearby to isoalloxazine ring of flavin cofactor that can be replaced by mutation without destabilization of the protein structure and which upon oxidation induced by irradiation triggers the dissociation of the flavin cofactor. We will test our hypothesis on selected flavoproteins such as flavodoxin, and NADH oxidase.

Aplication researh on utilization of lasers for remove of bacterial biofilms using combination of opto-chemical approaches.

supervisor: doc. RNDr. Gabriel Žoldák, PhD. (gabriel.zoldak@upjs.sk)
consultant: MUDr. Zuzana Hrabovská, PhD., JUHAPHARM, s.r.o. člen Cassovia Medi Valley z.p.o.
study form: full time

Annotation: The very recent increase in gastrointestinal infections is primarily due to biofilm-forming bacterial pathogens. The resistance of bacterial biofilms to traditional methods as well as the growing resistance to common antibiotics is a serious societal problem. The dissertation will aim to develop effective opto-chemical approaches that could locally remove battery biofilms, as well as to understand the detailed molecular mechanism of the proposed opto-chemical strategies. In addition to the use of standard known low molecular weight photosensitizers, we will also focus on the preparation and establishment of various types of photoactive proteins, such as the group of so-called synthetic infrared fluorescent proteins derived from bacterial phytochromes.

Molecular mechanism of the influence of laser radiation on the structure and composition of proteins.

supervisor: doc. RNDr. Gabriel Žoldák, PhD. (gabriel.zoldak@upjs.sk)
consultant: MUDr. Zuzana Hrabovská, PhD., JUHAPHARM, s.r.o. člen Cassovia Medi Valley z.p.o.
study form: full time

Annotation: By studying individual protein molecules, it has been observed that infrared laser radiation has a significant effect on the stability and dynamics of proteins and often leads to the inactivation of proteins and enzymes. The molecular mechanism of action of laser radiation includes indirect so-called mediated action on the conformation and structure of proteins. The dissertation will focus on understanding of the effect of laser radiation on the function and stability of human albumin and type I collagen proteins and the enzymatic activity of the matrix metalloprotease Mmp1. Understanding the molecular mechanism will make possible to design new research-based procedures to achieve a higher anti-aging effect.

Physico-chemical factors affecting the formation of light chain protein deposits in multiple myeloma.

supervisor: doc. RNDr. Gabriel Žoldák, PhD. (gabriel.zoldak@upjs.sk)
study form: full time

Annotation: Multiple myeloma is a severe cancer disease of plasma cells, which is often accompanied by the accumulation of large protein deposits consisting of light chain immunoglobulin IgG in vital organs of a man, e.g., heart, liver, and kidneys. At the molecular level, the rapid development of protein deposits has been associated with mutations at several positions in light chains. However, the role of external physico-chemical environmental factors in the tissue-specific development of protein deposits is poorly understood. This dissertation thesis will be focused on the analysis and classification of protein aggregates of several variants of the light chains in the presence of various physical perturbations (temperature, laminar flow) and chemical factors (pH change, ionic strength, and ion-specific effects). In a second step, we will test how cells interact with IgG light chain aggregates, and how these protein deposits impair function at the cellular level.

Singlet oxygen measurements in biological systems.
supervisor: doc. Mgr. Gregor Bánó, PhD. (gregor.bano@upjs.sk)
study form: full time

Annotation: Photodynamic therapy of cancer is based on the cytotoxic effect of singlet oxygen. Singlet oxygen is generated by energy transfer between photo-activated drug molecules (photosensitizers) and molecular oxygen. The range of singlet oxygen cytotoxic activity is determined by its diffusion rate and lifetime. Previous research in this area has not clarified the value of singlet oxygen lifetime in cells. The main objective of the PhD work is to develop an experimental apparatus for measuring the lifetime of singlet oxygen in cells and to obtain an answer to this key issue of photodynamic therapy.

Preparation and characterization of experiment for biological single particle imaging – rational design of samples (including start-to-end simulation of experiment.
supervisor: doc. RNDr. Jozef Uličný, CSc. (jozef.ulicny@upjs.sk)
study form: full time

Annotation: Imaging of single unique (i.e. non-repeatable and aperiodic) biological particles with high resolution in 3D faces several challenges and not every imaging modality is suitable for all objects. In hard X-ray imaging, the limited photon budget and the quality of imaging system form major bottleneck. By using extremely intense pulsed coherent sources – such as EuXFEL, it is possible to outrun the radiational damage of the sample as well as to dramatically increase the available photon budget. The tradeoff – so far – is the limitation to 2D single shot information, instead of getting full spatial information in single time slice. In our pilot experiments on EuXFEL (but also on 3rd generation synchrotron sources) we already demonstrated the feasibility of multiprojection imaging capable to lift up some limitations of projection imaging as well as to outrun radiational damage. The main topic of this PhD. work is – in close collaboration with experimental team - to make advantage of our methodological lead and to design specific experiments for imaging of biological particles of interest. This involves also design and execution of pilot experiments demonstrating feasibility of the approach. Besides the biological particles, we will make good use of microscopic size 3D-printed calibration objects co-designed by us and printed at partner institutions. The

theme also aims to explore the potential of machine learning to recognize the useful patterns of behavior and acceleration of data interpretation in high-throughput imaging experiments (up to MHz rates). Theme deals with computational design and simulation of experiment specifically for biological particles – e.g. DNA mesoscopic constructs (think origami) with high level of known *a priori* information – facilitating 3D reconstruction of imaging experiment. The task is to tailor the suitable class of artificial construct embedding fiducial particles with known affinity toward structural motifs on base of start-to-end simulation simulation of XFEL multiprojection experiment. The ultimate stage consists of synthesis and characterization of such particles. The theme is suitable for candidates interested in application of bioinformatics and statistical methods.

Preparation and realisation of experiment for single biological particle imaging - design of customised multiprojection experiment and its 3D reconstruction.
supervisor: doc. RNDr. Jozef Uličný, CSc. (jozef.ulicny@upjs.sk)
study form: full time

Annotation: Imaging of single unique (i.e. non-repeatable and aperiodic) biological particles with high resolution in 3D faces several challenges and not every imaging modality is suitable for all objects. In hard X-ray imaging, the limited photon budget and the quality of imaging system form major bottleneck. By using extremely intense pulsed coherent sources – such as EuXFEL, it is possible to outrun the radiational damage of the sample as well as to dramatically increase the available photon budget. The tradeoff – so far – is the limitation to 2D single shot information, instead of getting full spatial information in single time slice. In our pilot experiments on EuXFEL (but also on 3rd generation synchrotron sources) we already demonstrated the feasibility of multiprojection imaging capable to lift up some limitations of projection imaging as well as to outrun radiational damage. The main topic of PhD. thesis is – in close collaboration with experimental team - to make advantage of our methodological lead and to design specific experiments for imaging of biological particles of interest. This involves also design and execution of pilot experiments demonstrating feasibility of the approach. Besides the biological particles, we will make good use of microscopic size 3D-printed calibration objects co-designed by us and printed at partner institutions. The theme also aims to explore the potential of machine learning to recognize the useful patterns of behavior and acceleration of data interpretation in high-throughput imaging experiments (up to MHz rates). Theme is more oriented toward actual construction and eventual adaptation of actual experiment, where we expect the development of the apparatus [based on running in-house Eu-XFEL grant where we participate] from direct imaging techniques toward lensless coherent imaging where 3D reconstruction requires redesign/adaptation of existing algorithms, such as for inverse problem solutions. Topic is suitable for candidates with good background in physics – more intense communication with experienced beam scientists and on-site participation on apparatus building and experimentation under their mentoring is envisaged.

The study of NiR photobiomodulation effects in 2D and 3D cellular models of Parkinsons disease.
supervisor: doc. RNDr. Katarína Štroffeková, PhD. (katarína.stroffekova@upjs.sk)
study form: full time

Annotation: The etiology of the most neurodegenerations is not clear, however, interactions between genetic and environmental factors, lifestyles and dietary factors were shown to play a role in Parkinson (PD) or Alzheimer (AD) disease and ALS (amyotrophic lateral sclerosis). Long-term/low dose exposure to metals, pesticides, solvents, and petrochemicals were indicated as environment risk factors in PD, AD and ALS. PD was positively associated with two groups of pesticides, including rotenone (ROT) and paraquat (PAR), defined by mechanisms that impair mitochondrial function and those that increase oxidative stress further supporting a role for these mechanisms in PD pathophysiology. The ROT and PQ are used extensively in *in vitro* and *in vivo* PD models. Low-level near infrared therapy (NiR photobiomodulation, PBM) has a potential to fulfill neuroprotective and neuroregenerative tasks. For last 30 years, PBM effects were studied in wound healing, muscle repair, and angiogenesis. PBM has the ability to stimulate cell repair processes and proliferation. In last decade, mounting evidence appears to the beneficial effects of PBM in treatment of Parkinson and Alzheimer diseases, and in treatment of traumatic brain injuries including stroke. The present project aims to investigate PBM effects in 2D and 3D cellular PD model (rotenone challenged SH SY5Y cells). This study will focus on NiR effects on oxidative stress, Ca^{2+} signaling and a-synuclein changes in 2D and 3D cellular structures. The research will use an interdisciplinary approach using fluorescent microscopy, biochemistry, spectroscopy and molecular biology.

Institute of Experimental Physics SAS Košice

Biomedical lab-on-chip applications based on polymerized microstructures and their automation based on image analysis and machine learning principles.
supervisor: doc. Ing. Zoltán Tomori, CSc. (tomori@saske.sk)
consultant: doc. RNDr. Gregor Bánó, PhD., PF UPJŠ
study form: full time
Annotation: The trend of miniaturization aims to transform biomedical experimental techniques into “lab-on-chip” (LOC) applications. They often exploit the optical tweezers principle, where the laser beams drive the mechanical microstructures fabricated by two-photon polymerization and integrated into a microfluidic LOC environment. The main goal of PhD work is to automate LOC applications, where an intelligent autonomous algorithm controls the trajectories of manipulating laser beams according to the image analysis of the surrounding environment. In terms of experimental objectives, attention will be focused on two areas of LOC applications, namely microreology (measurement of the viscosity of the surrounding fluid environment based on deformation of elastic micro-springs) and the micromanipulation with particles (grabbing, transporting and releasing of the individual particles using the light-driven elastic micro-robots).

Modulation of protein amyloid aggregation – insight into molecular mechanisms of amyloid formation and inhibition.
supervisor: doc. RNDr. Zuzana Gažová, CSc. (gazova@saske.sk)
consultant: RNDr. Andrea Antošová, PhD.
study form: full time
Annotation: Amyloid structures of poly/peptides have been associated with diseases such as Parkinson's disease, systematic amyloidoses, diabetes mellitus and others. Recently, it has been found that amyloids are important for many essential processes

in organisms - from bacteria to humans. The aim of this work is to contribute to a better understanding of the mechanisms of the formation and inhibition of protein amyloid aggregation through their modulation by various substances (inorganic and organic molecules, biomolecules, nanoparticles). Based on this, it is possible to better understand the pathological and physiological effects of amyloid structures at the molecular level. Various physico-chemical methods will be used, mainly spectroscopic, calorimetric, chromatographic techniques and atomic force microscopy.

Misfolding proteins in amyloid diseases and ther prevention/therapy.
supervisor: doc. RNDr. Zuzana Gažová, CSc. (gazova@saske.sk)
consultant: RNDr. Zuzana Bednáriková, PhD.
study form: full time
Annotation: As our life expectancy increases, so does the likelihood of diseases such as Alzheimer's disease or diabetes. One of the causes of these amyloid diseases is an impaired synthesis of functional protein molecules and insufficient degradation of non-functional, misfolded protein molecules. As a result, misfolded proteins accumulate in the form of amyloid aggregates with a high content of β-sheets in various human body tissues. There is currently a lack of detailed knowledge of the causes of amyloid formation and no treatment for any known amyloid diseases. We will use modern biophysical methods to study the mechanisms of amyloid aggregates formation of globular and intrinsically disordered proteins associated with diseases such as AD, diabetes or systemic lysozyme amyloidosis. At the same time, we will focus on the systematic search for interaction partners to prevent these diseases, respectively treat them.

Protein-based anomaterials- biochemical and biophysical evaluation.
supervisor: RNDr. Ing. Katarína Šipošová, PhD. (siposova@saske.sk)
consultant: doc. RNDr. Erik Sedlák, DrSc. – CIB TIP UPJŠ
study form: full time
Annotation: Amyloid fibrils are chiral protein-based systems, formed through the self-assembly of β-sheet aggregates into twisted or helical ribbons. It is generally accepted that all peptides and proteins could be transferred into the amyloid state, if appropriate conditions are applied. In addition, amyloid structures hold great potential to be used for the preparation of molecular nanomaterials (e.g. nanowires, nanolayers, gels, scaffolds, templates, and liquid crystals) using the “bottom-up” strategy due to their structural compatibility, nanoscale dimensions, efficient assembly into well-defined ultrastructures, ease of production, and low cost.To reveal their potential, the proposed PhD thesis is aimed at the determination of the conditions leading to the controllable "bottom-up" formation of amyloid fibrils, describing the mechanism of amyloid formation and morphology of the formed fibrils. Utilization of the controllable self-assembly of amyloidogenic proteins is planned to establish a reliable method for the preparation of amyloid-based nanocomposites. The main task will be: (1) establishing of robust production of amyloid fibrils at mild conditions, (2) immobilization of proteins on/in amyloid assemblies; (3) characterization of amyloid-based nanocarriers for controlled drug delivery; (4) utilization of biochemical and biophysical methods to characterize properties of immobilized proteins and amyloid-nanocomposites as a whole.

Protein stability and aggregation in biocompatible organic solvents.

supervisor: RNDr. Diana Fedunová, PhD. (fedunova@saske.sk)

study form: full time

Annotation: The identification of effective solvents capable of modulating protein stability and aggregation is of great importance for various applications in biotechnology or medicine. The production and long-term storage of proteins require setting appropriate environmental conditions that preserve the native structure of the proteins and prevent their aggregation. Similarly, the formation of a special type of ordered aggregates - amyloid fibrils, is conditioned by external conditions. Amyloid aggregates represent new potential biomaterials due to their unique properties (strength, stability, elasticity, resistance to degradation). Therefore, finding conditions capable of inducing the formation of defined amyloid aggregates is of interest. This work aims to study the effect of special solvents - ionic liquids and deep eutectic mixtures - on the stability, kinetics of amyloid aggregation, and morphology of amyloid fibrils of various proteins (lysozyme, insulin). The objective is to determine the relationship between the composition and physicochemical properties of solvents and their ability to stabilize/destabilize protein structure and inhibit/accelerate amyloid aggregation to find solvents capable of stabilizing studied proteins or inducing amyloid aggregate formation with defined morphology. Spectroscopic (UV-VIS, CD, FTIR) and calorimetric (DSC, ITC) methods, as well as atomic force microscopy (AFM) and computer image analysis methods, will be used.